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Systematic Review

Extinction Status, Challenges, and Conservation Approaches of South African Indigenous and Village Chickens: A Systematic Review

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Abstract: South Africa recognizes the value of indigenous breeds such as Potchefstroom Koekoek, Boschveld, Ovambo, Venda, Naked Neck, and nondescript village chickens. Indigenous chickens support sustainable food systems, improve nutrition, and enhance livelihoods in rural communities, thereby contributing to the United Nations' Sustainable Development Goal (SDG) 2 on Zero Hunger. These breeds are not only vital to rural farmers for food production, income generation, and subsistence but also provide rural farmers with cheap nutritious protein such as eggs and meat for household consumption. Moreover, they are preferred by rural farmers because they are relatively affordable to produce, can withstand harsh environmental conditions amid accelerated climate change compared to exotic breeds, and require less/no feed supplementation. However, despite the numerous advantages of keeping these chickens, it has been found that they are mostly in danger of extinction due to evolving production methods that favor exotic breeds. Therefore, understanding their extinction status, different implications for conserving their genetic material, challenges encountered, and future approaches to rescue these breeds remain vital. Hence, the aim of this systematic review was to assess the extinction status, challenges, and conservation approaches for these breeds. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines were utilized to search for suitable articles addressing the objective of the current review. Research articles were grouped and evaluated for eligibility, and the data from the Domestic Animal Diversity Information System database were used. Records such as duplicates of studies addressing origins, phenotypic and genetic diversity, the conservation of indigenous chickens, semen cryopreservation of indigenous chickens, climate change effects on indigenous chickens, and the use of extenders with exotic chickens and other chicken types, reports in other languages, and reports that were inaccessible were excluded. Articles addressing origins, phenotypic and genetic diversity, the conservation of indigenous chickens, semen cryopreservation of indigenous chickens, climate change effects on indigenous chickens, and the use of extenders with indigenous chickens were included in this review. The keywords used to search articles online were as follows: South African indigenous chicken; extension status; conservation; genetic resources; genetic markers; effective population size; inbreeding; and characterization. This systematic review found that there is less information in the Domestic Animal Diversity Information System regarding South African indigenous and village chickens, suggesting a lack of reporting in this system. Moreover, our review confirmed that most South African indigenous chickens are threatened and, hence, require interventions such as assisted reproductive technologies and other strategies in order to improve efficiency.

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1. Introduction

The United Nations' Sustainable Development Goals (SDGs) and the African Union's Agenda 2063 call for food security in all households and poverty alleviation [1]. Nevertheless, the majority of South Africans, particularly those living in rural areas, continue to live below the breadline [1]. As a result of the COVID-19 pandemic and a weakening economy, poverty in rural areas has intensified to about 45% in comparison to those living in urban areas, sitting at 19% [1,2]. This has been accelerated by a rising youth unemployment rate in South Africa (+33%) [3]. Globally, South Africa is amongst the Global South countries most affected by climate change, and this is occurring during a period of exponential population growth in the country and worldwide [4]. All these factors will necessitate or force farmers to practice subsistence farming to curb hunger, food insecurity, and poverty. Moreover, with these facts in mind, it appears that amongst the other South African indigenous breeds, indigenous chickens have a fundamental role in food security for the rural population [5,6]. There are strategies in place to mitigate climate change, including the use of indigenous breeds that are adapted to the local environment. These breeds are characterized to have traits that assist them to survive and adapt to climate change such as light coat color, less subcutaneous fat, increased sweating and respiration rate, increased pulse rate, reduced metabolic rate, more water intake, and an altered expression of heat shock [7]. Their adaptation is also linked to the fact that they have typical genetic development while also having a strong ability to sense danger and act fast [8]. Due to the existence of several key genes (e.g., HSP70 and HSP90) in their gene pool that protect them from the harmful effects of heat stress, indigenous chickens are better suited to tropical environments [9].

South African-recognized indigenous chickens include Potchefstroom Koekoek, Naked Neck, Ovambo, Boschveld, Venda, and nondescript village chickens [8]. It has been reported that these chickens are mostly preferred by rural farmers because they are hardy, well-adapted to harsh environments, and require fewer veterinary interventions [8,10]. They are important to rural farmers because they offer inexpensive protein substitutes like meat and eggs, which is why they are essential to food security. However, there have been reports that some breeds are in danger of going extinct [8,11]. This is because of challenges such as inbreeding, which occur due to a lack of education and knowledge provided to rural farmers, poor housing, a lack of coordinated disease-control mechanisms, parasitism in the intestines, poor feeding, and the absence of conservation strategies [8]. It is known that the significance of animal genetic diversity should be protected since there is a chance that the goals of livestock production and performance, as well as the production conditions, will change significantly in the future [12]. This can be accomplished through improved breed characterization, improved systems for detecting and reducing signals of genetic diversity, successful in situ and ex situ conservation efforts, genetic improvement programs targeted at improving outcomes and performance traits in locally adapted breeds, increased support for countries that are developing the management of their animal genetic resources, and easier access to genetic resources and related knowledge [12]. Traditionally, most of the rural farmers (owning 88.89% indigenous cockerels) practice uncontrolled breeding and do not share cockerels amongst themselves to avoid genetic wastage and improve conservation gains. Rather, they would prefer donating or slaughtering the cockerels if they are no longer of use to them, which then leads to extinction [13]. In general, when the breed is endangered, assisted reproductive biotechnologies (ARTs) such as artificial insemination (AI) remain vital for the improvement of such breeds for conservation purposes [11,14].

Artificial insemination is a recently developed ART that has been used to conserve the genetic materials of mammals, including semen and embryos, successfully [15]. These ARTs offer different ways to increase reproductive performance and later profit while drastically reducing the unwanted costs, such as that of rearing and feeding males in the flock [16]. The mounting significance of AI in poultry reproduction has caused a spiking interest in developing the proper conditions for the preservation of semen [17]. It would be possible for poultry breeders to use superior males and inseminate many females, even on isolated farms, if semen from chickens can be diluted and stored. However, the reduced sperm motility survival after the frozen–thawed procedure makes cryopreservation of cockerel semen difficult [18]. Additionally, advancements have been made in the development of cryopreservation procedures and semen diluents for poultry semen [19]; however, the survival and motility rates remain poor [18]. Therefore, this systematic review is aimed at determining the extinction status of South African indigenous and nondescript village chickens, the obstacles to their successful conservation, and the conservation strategies currently being employed to preserve these species.

2. Methodology

This review's goal was to discuss the extinction status, difficulties, and conservation strategies of indigenous and village chicken breeds in South Africa. The characterization of studies included on the results are displaced in Table 1 below. In this review, information was collected, evaluated, and exploited from the Domestic Animal Diversity Information System (DAD-IS) database, as well as relevant publications from academic databases such as ScienceDirect, PubMed, Scopus, TUT online library, Semantic Scholar, and ResearchGate. The following keywords were used to look for articles online: indigenous chickens; conservation; fertility; genetic improvement; reproductive performance; and artificial insemination. To find articles and information suitable for the content of this review, the search was limited to articles from 2000 to 2024 in academic paper databases. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines were then incorporated after a thorough review of all articles, as described by [20] and illustrated in Figure 1 below.

Table 1. Characterization of South African studies included in the results of this review after screening using DAD-IS and academic paper databases.

Publication Year	Breed Studied	Studies
2024	Naked Neck, Venda, Potchefstroom Koekoek	[21]
2010	Naked Neck, Venda, Ovambo, Potchefstroom Koekoek, Boschveld	[22]
2009	Ovambo, Potchefstroom Koekoek	[23]
2022	Naked Neck, Venda, Ovambo, Potchefstroom Koekoek, Boschveld	[24]
2009	Potchefstroom Koekoek	[25]
2023	Venda	[18]
2009	Ovambo, Potchefstroom Koekoek	[19]
2020	Naked Neck, Venda, Ovambo, Potchefstroom Koekoek, Boschveld	[8]
2016	Venda	[26]

Table 1. *Cont.*

Publication Year	Breed Studied	Studies
2011	Naked Neck, Venda, Ovambo, Potchefstroom Koekoek	[27]
2011	Naked Neck, Venda, Ovambo, Potchefstroom Koekoek, village chickens	[28]
2016	Venda, Ovambo, Naked Neck, Potchefstroom Koekoek, village chickens	[29]
2006	Venda	[30]
2006	Venda	[31]
2020	Potchefstroom Koekoek, Ovambo, Venda	[32]
2017	Boschveld	[10]
2008	Naked Neck, Venda, Potchefstroom Koekoek, Ovambo	[33]
2024	Potchefstroom Koekoek, nondescript village chickens	[34]

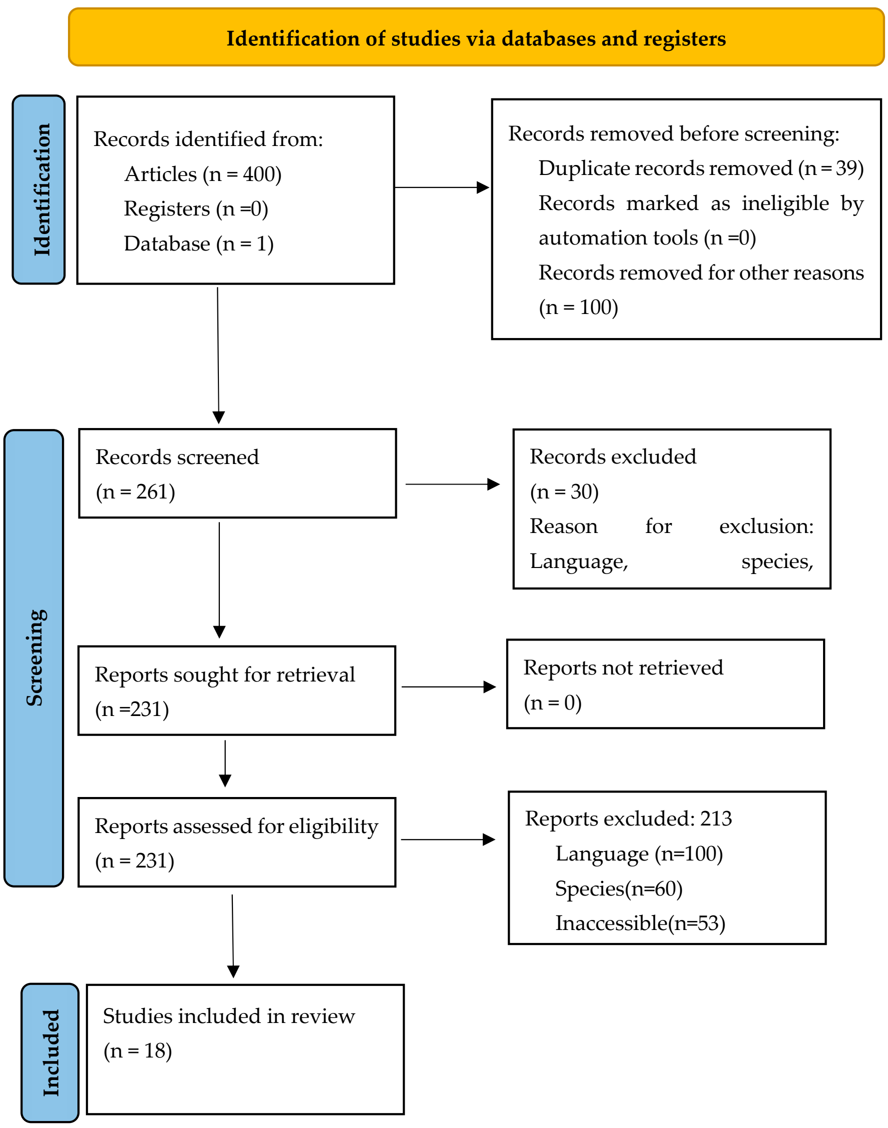

Figure 1. PRISMA flow diagram [20].

3. The Literature Search

All articles concerning South African indigenous chickens were gathered using an online database search.

1. South African indigenous chicken (origin);
2. South African village chickens;
3. Extension status of South African indigenous chickens;
4. Effective population size of indigenous chickens;
5. Conservation status of South African indigenous chicken breeds;
6. Reproductive performance of indigenous chickens (semen characterization);
7. Genetic diversity of South African indigenous chickens;
8. Phenotype characterization of indigenous chicken breed;
9. Use of extenders in semen preservation of chickens.

These terms were used in Google Scholar, ScienceDirect, PubMed, Scopus, TUT online library, Semantic Scholar, and ResearchGate, and the databases were used to search for suitable articles. Only articles published between 2000 and 2024 were considered. Only articles written in English were considered for the next step; those in other languages were excluded.

Exclusion and Inclusion Criteria

Records identified in the process of writing this review consisted of 400 articles and 1 database. For the quantitative assessment, preference was given to studies that were either a randomized experiment or used a quasi-experimental method. The qualitative assessment was conducted to select studies that met two criteria: (1) studies with clear research objectives that were relevant to the current review; and (2) studies that provided sufficient information related to the context, sample selection, and data collection procedures. Records such as duplicates (39) and studies addressing origins, phenotypic and genetic diversity, the conservation of indigenous chickens, semen cryopreservation of indigenous chickens, climate change effects on indigenous chickens, and the use of extenders with exotic chickens and other chicken types, reports that were in other languages, and reports that were inaccessible were excluded (213). Peer-reviewed articles published between 2000 and 2024 were selected and used in this review. Articles addressing origins, phenotypic and genetic diversity, the conservation of indigenous chickens, semen cryopreservation of indigenous chickens, climate change effects on indigenous chickens, and the use of extenders with indigenous chickens were included in this review. Moreover, 18 articles were considered for this review and were strictly selected based on the country they were from (South Africa).

4. History and Characteristics of South African Indigenous and Nondescript Village Chickens

Common indigenous chicken breeds in South Africa include the Potchefstroom Koekoek, Ovambo, Boschveld, Naked Neck, and Venda. Despite the limited information on these breeds, the majority of them are frequently defined and categorized according to their geographic location or phenotypic traits [33]. These indigenous chickens typically differ in terms of plumage color, body size, feather patterns, comb forms, and shank color. They are often harder and more climatically adapted to South Africa, making them more suitable for small-scale and free-range production when compared to commercial breeds [22]; hence, they are characterized by their low input, low output system [8]. Indigenous chickens have been reported or seen across South Africa according to the ecosystems of their characteristics [8]. For example, researcher Marais crossed White Leghorn, Black Australorp, and Barred Plymouth to make the indigenous Potchefstroom Koekoek chicken breed, which was produced in the 1950s at Potchefstroom Agricultural College [22,35]. It is said to outperform the majority of indigenous chickens in South Africa [24]. This breed is able to survive in tropical regions with hot climatic conditions. The Koekoek name signifies the barred color pattern of the chicken. This type of chicken is raised for both meat and eggs, and has a high degree of adaptability to living in a free-range environment [24].

Ovambo is described as a small-framed breed of chicken from Ovambo land in Africa and the northern section of Namibia [8,24,35]. A researcher who visited Namibia's Ovambo Land District in 1975 saw this Ovambo breed and collected some of them to start a breeding colony and keep it from going extinct; hence, some of these chickens are now being conserved at the Agricultural Research Council in Irene in the Poultry Breeding Unit [22]. This breed is recognized for its aggression, agility, and dark to black feathers with white or orange stripes [22], which assist in camouflaging the bird to protect it from predators, making it known as a suitable breed for rearing among rural chicken farmers [36]. Furthermore, out of all the indigenous breeds of chickens in South Africa, Ovambo chickens have the largest dressed carcass mass [36]. According to [36], these advantages make the Ovambo breed highly desirable for meat purposes, while [24] distinguishes their genotypes as layers that are susceptible to harsh conditions, with an average body weight of 1.32 kg at 16 weeks and 1.54 kg at 20 weeks.

The Venda chicken was named after the former Venda province—which is now a part of the province of Limpopo in South Africa—and was initially described by veterinarian Dr. Naas Coetzee [31]. Venda chickens are colorful, ranging from black, white, and red, with rose-colored combs and five-toed feet dominating the population. The breed is characterized as large, and they lay large, though tinted, eggs at an average of 70 eggs yearly, weighing about 53 g [28]. Venda chickens have good nurturing capacity, broodiness, superior survivability, can withstand harsher environmental conditions, and are tolerant to some diseases [30]. These chickens are able to scavenge, making them capable of maintaining themselves without being fed. They can feed on many sources of food such as household leftovers, seeds, flying and soil-based insects, lizards, and small rodents [37].

Boschveld, on the other hand, is an indigenous chicken genotype that was acquired through combining Venda, Matabele, and Ovambo indigenous chickens [10]. This chicken is good at maintaining itself, can walk longer distances in search of food for survival, and is well-adapted to extreme environmental conditions [24]. According to [38], Boschveld chickens are disease-tolerant, grow quickly, and function well in scavenging environments and on a free-range system where household leftovers are used for feeding. This chicken has a large body frame, is hardy, and encompasses a mixture of brown and white feathers.

Although the Naked Neck chicken breed's origins are unknown, early traders from Malaysia likely brought the breed when traveling across the continent [8,22]. These indigenous chickens are split into two categories: purebred birds, which have a completely naked neck; and non-purebred birds, which have a tassel on the front of the neck [24]. The variety of color patterns of the chicken aids in its ability to camouflage and shield itself from predators on the ground [39]. Naked Neck chickens are resilient to harsh environments and can survive by scavenging for food [8,24].

Nondescript village chickens are those kept and reared in rural communities [40]. They play major roles in these communities, such as providing cheap protein such as eggs and meat, vital for income generation through sales [41], and are used for traditions and rituals in different ethnicities [40]. They can be raised extensively, however, a flock size of less than 100 is common and they depend largely on scavenging for feed [40]. Furthermore, these village chickens have significant economic, social, and nutritional roles while financially empowering women by supporting livelihoods in their households. Additionally, they are essential to sustainable agriculture since they help control insects and pests in crops by eating pests, tilling, foraging, and offering natural fertilizers by means of manure, which is rich in nutrients (nitrogen, potassium, phosphorus, and calcium) and can improve soil structure, moisture retention, and drainage [42], reducing the need for chemical pesticides and fertilizers [34].

5. Role of Indigenous Chicken Breeds in the Rural Economy and SDG 2—Zero Hunger and Poverty Alleviation

In developing countries like South Africa, chickens offer a variety of uses and advantages for households [43]. The use of indigenous chickens in the tropics differs from place to place and from community to community [9,38]. For instance, some farmers in these regions use chickens for religious purposes [38] due to a person's or their community's devotion to a certain spiritual being, the season, and customs and/or religious ceremonies. The chickens are judged based on the caliber of the offering, and whether it meets the recipient's specific requirements for the chicken's morphological features [43,44]. They are an essential component of a reliable supply of high-quality animal protein, which is also significant to the sociocultural life of rural communities [45]. For instance, the entire world produced 87.0 million tons of chicken eggs and 139.2 million tons of chicken meat in 2022, which contributes to high-quality protein [46].

Furthermore, during the day, chickens typically wander around the household yards, where they consume leftover food items such as grains, legumes, and rice, as well as insects and other readily available nutritional sources [22,43]. These chickens then convert waste foodstuff to provide a good quality, affordable source of animal protein [43]. Thus, they are known to make further contributions to rural communities by supplying eggs and meat suitable for human consumption and as a source of income [47]. However, they are said to have slow growth performance and are categorized as poor layers, laying small-sized eggs. Nevertheless, they have a good mothering ability and are excellent brooders, foragers, hardy, and have natural immunity against common diseases [43]. They are well-known for their hardiness and suitability for enduring harsh environmental conditions, a lack of veterinary services, and having fewer water and feed requirements [8]. In a rural setup, chickens are mainly reared by women and youth, generating cash revenue or emergency cash and supplying eggs and meat for consumption [43].

6. South African Indigenous Chicken Breeds' Current Status and Extinction Risks

An early study estimated that about 33% of indigenous chicken breeds are in danger of extinction due to the evolving production methods that favor exotic commercial breeds and are not interested in crossbreeding; therefore, many indigenous breeds continue to lack distinctive qualities (characterization) [8,48]. Moreover, the majority of the poultry world population, which is composed of indigenous breeds (about 80%), is unknown to scientists, and 40% of them have an unknown extinction status [9]. The Global Plan of Action for Animal Genetic Resources launched by the FAO was developed to characterize and conserve indigenous breeds to curb the irreparable degradation of animal germplasm that could jeopardize potential breeding programs. Moreover, countries such as Uganda have launched biobanking training for new initiatives to preserve Africa's indigenous chickens [49]. China, on the other hand, has established both in situ and ex situ programs for the management of poultry genetic resources, as per the regulations issued by the Ministry of Agriculture and Rural Affairs [50]. From these programs, a study by [50] not only provided a valued reference for evaluating the current conservation chicken programs in China but also gave evidence that these are more effective than in other countries because they have maintained the genomic diversity of the studied indigenous chickens. South Africa also has a conservation program, "Fowls for Africa", established by the Animal

Production Institute of the Agricultural Research Council (ARC-AP) in 1994 as a base for conservation and genetic improvement [29]. This emphasizes the need for conservation in order to fight against the extinction risks of indigenous chickens. However, it is our view that there is little to no information and a lack of reporting on the population data of South African indigenous breeds like Potchefstroom Koekoek, Ovambo, Boschveld, and nondescript village chickens (Table 2). These data are essential for the effective structuring of conservation initiatives and encouraging the use of indigenous chicken genetic resources in commercial production. Hence, the Agricultural Research Council established a breeding program in 1994 with indigenous breeds such as Venda, Naked Neck, Ovambo, and Potchefstroom Koekoek [29]. Still, the protection of these chicken breeds over extended periods of time may be jeopardized because protection choices for these indigenous chickens were primarily based on common population development parameters like reproduction and production, with less consideration for genetic factors posing a threat of extinction. Van-Koster et al. [33] revealed high Fis estimates (0.21–0.35) and moderate genetic diversity with the corresponding breeds (He = 0.50–0.65), which may indicate inbreeding within the breeds. On the other hand, Mtileni et al. [27] revealed that the heterozygosity estimates were similar (He = 0.51–0.62) between the two investigations, while the Fis estimates ranged from −0.048 to 0.041. Thus, evaluating the probability of extinction and conservation limits of individual populations using the effective population size as a criterion was incorporated to find mitigating strategies that may prevent genetic erosion and the disappearance of these chickens. To evidence this initiative, Mtileni et al. [29] conducted a study on the conserved flock's (Potchefstroom Koekoek, Ovambo, Naked Neck, and Venda) effective population size and reported point estimates ranging from 38.6 to 78.6 (Table 3). To curb these challenges, initiatives to employ ARTs such as the cryopreservation of indigenous breeds' genetic resources and germplasm enriched with multinutrients (antioxidants and dietary supplements to improve fertility) should be kept in place; moreover, breeding practices should be centered around using AI and males that will be continuously monitored through genetic diversity so as to avoid a rise in inbreeding and a declining population size.

Table 2. Extinction status of South African indigenous chickens [21].

Population Data	Potchefstroom Koekoek	Venda	Ovambo	Boschveld	Naked Neck	Nondescript Village
Reporting year	-	1994	-	-	1994	-
Population trend	-	Increasing	-	-	Increasing	-
Breeding hen	-	120	-	-	200	-
Breeding cockerel	-	40	-	-	100	-
AI performed	-	Yes	-	-	-	-
Cockerels in AI	-	0	-	-	0	-
Extinction status	Unknown	Unknown	-	-	Unknown	-

Table 3. Approximations of Ne using heterozygosity.

Population	Effective Population Size	
	Heterozygosity Excess	Linkage Disequilibrium (95% CI)
OV_C	∞	78.6 (43.0–2661)
PK_C	∞	47.9 (30.3–96.2)
NN_C	8.9	38.6 (25.9–66.9)
VD_C	46.5	42.2 (26.0–85.5)

Adapted from [29] with permission from Prof. Bohani Mtileni from Tshwane University of Technology, South Africa. N.B. (to be noted): OV_C—Ovambo conservation; PK_C—Potchefstroom Koekoek conservation; NN_C—Naked Neck conservation; VD_C—Venda conservation

7. Phenotype and Genetic Characterization

Genetic and phenotype characterizations have been used as conservation techniques to classify and describe indigenous species for a better understanding of the breeds and to improve the successful application of technologies. Phenotypic characterization can be defined as the procedure of recognizing several populations of breeds and characterizing their production and external traits in a specific production setting [51]. These traits (phenotypes) include visible traits such as height, weight, eye color, and hair color. Most of these South African indigenous chicken breeds have been characterized by appearance in several studies (Table 4); for instance, Naked Neck chickens were characterized by [8,24] as chickens that are very colorful, with plain heads and single comb, having medium-sized wattles in females and favorably developed wattles in males.

Table 4. Phenotypic characteristics of South African indigenous chickens.

Phenotypic Characterization	Potchefstroom Koekoek	Venda	Ovambo	Boschveld	Naked Neck	Nondescript Village	Reference
Head	Black, white, red	Rose-colored combs	Black, orange	Brown and white	Plain head and single comb	-	[8]
Average body size (hen)	2.5–3.5 kg	2.0 kg	1.54 kg	2.5–3 kg	2.0 kg	2.94 kg	[34,40,41]
Average body size (cockerel)	3–4 kg	1.9 kg	2.16 kg	1.6–2.6 kg	1.6 kg	3–4 kg	[15,41]
Coat color	Light and dark gray with white barring	Black, white and black	Black, white, and orange	Light red–brown and white	Black and brown	Any color	[32,38,41]
Comb type	Single	Single	Single	Single	Single	Any color	[30,34,52]
Comb color	Red	Red	Red	Red	Red	Any color	[30,34,52]
Egg shell color	Brown	Tinted	Brown	Brown	Light brown	-	[30,32]
Egg production	198 p.a.	129 p.a.	129 p.a.	200 p.a.	138 p.a.	-	[22,24]
Growth rate	-	-	-	-	-	-	[21]

Additionally, their production traits have been described as having improved egg output and quality, resilience to disease, higher weight gain and dressed percentage, and tolerance to extreme ambient temperatures. Ovambo chicken has been characterized as having feathers of a dark to black color, with stripes of white and or orange [35], and has high egg and meat production [22]. On the other hand, Potchefstroom Koekoek has been characterized as having feathers that are light and dark gray with white barring [8] and they are known to lay brown-shelled eggs with an average weight of 55.7 g, with a hatchability rate reaching up to 78%.

Venda and Boschveld chickens have also been characterized phenotypically, as shown in Table 4 [8,21]. However, it is stated that nondescript breeds are closely related to chickens that vary in weight, body form, color, and comb type, and that may or may not have shank feathers [34].

Notably, all these breeds are characterized to have high egg production rates, ranging from 129 to 200 eggs per annum. This might be due to genetic potential as well as the way in which their feed is composed [22]. Moreover, there is no information or data recorded on the DAD-IS system on the growth rate of these chickens, which continues to be a concern (displaying a lack of reporting). Such systems are important for providing access to searchable databases for breed-related information and further monitoring of national breed populations to assist in making informed decisions on the management of animal genetic resources.

Genotypic characterization of indigenous chicken breeds—which entails the process by which accessions are identified or differentiated in the appearance or make-up of an individual—has been performed in South African indigenous chickens using microsatellite markers (Table 5). Understanding how the population is structured and the genetic diversity distribution in livestock is crucial for enhancing selection strategies and breed development, as well as for promoting biodiversity conservation, optimizing breed utilization, and implementing tailored conservation programs based on local conditions [32].

Table 5. Characterization of South African indigenous chicken breeds' genetic diversity using microsatellite markers.

Type of Breed	Sampling Area	Ho	He	MAF	Fis	*p*-Value	References
Potchefstroom Koekoek	Dairy and Beef Research Institute, Irene	0.36	0.50	-	0.28	-	[33]
Potchefstroom Koekoek	KwaZulu-Natal	0.50	0.51	-	-	-	[32]
Potchefstroom Koekoek	ARC	0.53	0.53	-	0.005	-	[27]
Potchefstroom Koekoek	Glen Poultry State Farm	7.15	-	-	-	-	[23]
Naked Neck	Dairy and Beef Research Institute, Irene	0.48	0.65	-	0.26	-	[33]
Naked Neck	ARC	0.60	0.57	-	0.048	-	[27]
Venda	Dairy and Beef Research Institute, Irene	0.42	0.53	-	0.21	-	[33]
Venda	KwaZulu-Natal	0.53	0.59	-	-	-	[32]
Venda	ARC	0.51	0.51	-	0.002	-	[27]
Ovambo	Dairy and Beef Research Institute, Irene	0.40	0.62	-	0.35	-	[33]
Ovambo	ARC	0.59	0.62	-	0.041	-	[27]
Ovambo	Glen Poultry State farm	8.43	-	-	-	-	[23]

N.B. (to be noted): Ho—observed heterozygosity; He—expected heterozygosity; MAF—minor allele frequencies; Fis—inbreeding coefficient; ARC—Agricultural Research Council.

Van Marle-Köster et al. [33] reported the genetic diversity, which is an unbiased heterozygosity He, ranging from 0.65 in the Naked Neck population to 0.50 in the Potchefstroom Koekoek, with an average genetic diversity of 0.56. Nxumalo et al. [32] reported mean values of H_O and H_e at 0.59 and 0.63 (above 50), respectively, which highlights noticeable genetic diversity across the studied chicken population. According to [27], the continuing gene flow as a result of immigration and emigration amongst subpopulations may be the reason why there is an increased genetic diversity that is influenced by scavenging populations sharing the same geographic zone. Additionally, a clear subdivision between the conservation flocks (Potchefstroom Koekoek, Venda, Ovambo, and Naked Neck) and village chicken populations was reported by Mtileni et al. [27]. Furthermore, it was detected that the most likely grouping occurred at K = 5. This resulted in the four conservation flocks being categorized as separate clusters, with the three chicken village populations forming a single cluster regardless of the K-value. The segregation of the conservation and village chicken flocks into distinct clusters suggests variations in allele frequencies between these two population groups.

The genetic diversity of the nondescript village chickens was evaluated, as outlined in Table 6 for the different provinces. Mtileni et al. [27] reported an Ho that ranged from 0.61 to 0.64 and He ranging from 0.67 to 069, while in the KwaZulu-Natal province, Nxumalo et al. [32] reported an Ho of 0.61–0.70 and an He ranging from 0.65 to 0.72.

Table 6. Genetic diversity of nondescript village chicken populations.

Population	Sample Size	Ho ± SD	He ± SD	Fis	References
LP	42	0.63 ± 0.01	0.68 ± 0.02	0.075	[27]
NC	30	0.61 ± 0.02	0.67 ± 0.02	0.085	[27]
EC	26	0.64 ± 0.02	0.69 ± 0.02	0.066	[27]
JO	30	0.70 ± 0.02	0.68 ± 0.03	−0.030	[32]
NE	30	0.67 ± 0.02	0.65 ± 0.02	−0.038	[32]
PM	30	0.61 ± 0.02	0.66 ± 0.02	0.074	[32]
PO	30	0.68 ± 0.02	0.72 ± 0.02	0.067	[32]

N.B. (to be noted): LP—Limpopo Province; NC—Northern Cape; EC—Eastern Cape; JO—Jozini; NE—Newcastle; PM—Pietermaritzburg; PO—Port Shepstone; Ho—observed heterozygosity; He—expected heterozygosity; Fis—inbreeding coefficient.

The genetic diversity of indigenous chicken breeds has been further evaluated using mitochondrial DNA markers (Table 7). Mtileni et al. [28] reported a 12.34% genetic variation between the South African-maintained populations (Potchefstroom Koekoek, Naked Neck, Ovambo, and Venda), whereas within-population diversity was 87.66% of the total variation. In addition, a bigger 76% within-population variation in the total maternal variance has been observed [32]. Overall, phenotypic characterization of indigenous breeds has been a successful task considering the abovementioned results. This will assist and educate farmers in understanding different population breeds and their production traits, emphasizing the importance of maintaining breed purity to reduce genetic loss. Moreover, the results showed Ho and He values that are above 50, which highlights noticeable genetic diversity across the studied chicken population. Therefore, understanding genetic diversity assists species in adapting to environmental changes, reduces the risk of inbreeding, and supports ecosystem function, which are valuable factors that will help in ensuring that indigenous breeds' germplasm is conserved in situ and ex situ to mitigate challenges like extinction.

Table 7. Genetic diversity of South African indigenous chicken breeds distinguished using mitochondrial DNA.

Source of Variation	Sampling Area	Sum of Squares	Variance Component	Variance (%)	References
Between populations	KZN	18.185	0.15462	5.24	[32]
	ARC	40.234	0.31909	12.34189	[28]
	ARC	165.96	0.04	0.57	[29]
Within populations	KZN	226.387	2.24146	76.00	[32]
	ARC	237.965	2.26633	87.65811	[28]
	ARC	260.5	6.26	88.92	[29]

N.B. (to be noted): KZN—KwaZulu-Natal; ARC—Agricultural Research Council.

8. Conservation Approaches and Progress

Conservation strategies that can be used in order to protect valuable genes include "in situ" conservation, which can be described as conservation occurring in the natural habitat or conserved within natural populations of plant or animal species. It is known for allowing species to thrive in their natural environments, maintains ecological interactions and evolutionary processes, is cost-effective, and encourages public participation in conservation efforts. In situ conservation also has its own limitations—such as but not limited to insufficient space for animals—which might cause a substantial decrease in genetic diversity. Ex situ conservation, on the other hand, entails preserving threatened species, varieties, or breeds of plants or animals outside their natural habitats through in vitro or in vivo methods (Figure 2). In vivo conservation typically takes place in a controlled setting, while in vitro preservation focuses on storing gametes and utilizing advanced reproductive biotechnologies [15]. Ex situ conservation strategies are known to preserve genes and gametes, which can be used for future studies, can improve the chances of successful breeding—through the use of ARTs such as embryo transfer and IVF—and aid in the reintroduction of conserved species into the population. However, this conservation method has limitations, such as possible altered genetic diversity during selection, nutritional issues, diseases, changes in behavior that can influence reproduction, financial limitations, and possible problems with isolating, cloning, and transferring genes. Thus, conservation of valuable animal germplasm that remains accessible for forthcoming use by chicken breeders is one of the Global Plan of Action's strategic priority areas.

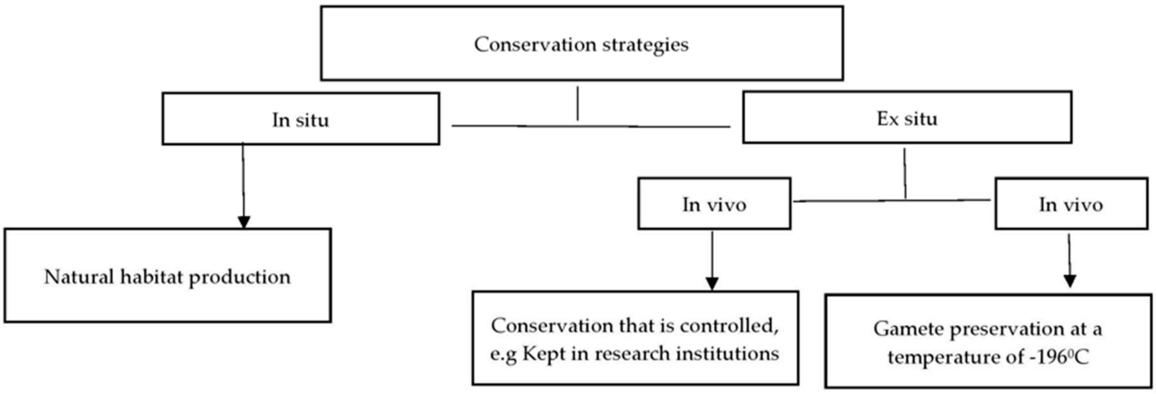

Figure 2. Different types of livestock conservation [15]. Adapted from [15], with permission from Dr. Jabulani Ngcobo from Tshwane University of Technology, South Africa.

In situ and ex situ conservation approaches are corresponding techniques widely used to preserve species; thus, merging both methods can be a vital conservation strategy. Gamete or embryo cryopreservation in vitro in a gene bank is the most well-known type of ex situ conservation. The conservation of gametes or material ex situ (in vivo and in vitro) has been a dynamic method to prevent breeds from becoming extinct. For instance, Ovambo chickens are conserved by the South African Stud Book and Livestock Improvement Association on site (Table 8). However, there seems to be a lack of reporting or little information recorded on databases like DAD-IS on the data of institutions that are keeping these animals on site, e.g., the Agricultural Research Council has been reported to have kept Ovambo chicken on site for conservation purposes [22]. Moreover, there seems to be no information available or recorded on the conservation of nondescript village chickens in this system. A lot of research studies have been conducted using indigenous chickens conserved at ARC Animal Production and the Dairy and Beef Research Institute, Irene [18,26,27]. It is of high importance for this data to be reported on such platforms to be able to improve conservation knowledge and track what exists and what does not.

Table 8. Conservation of South African indigenous chicken breeds in vivo [21].

Population Data	Potchefstroom Koekoek	Venda	Ovambo	Boschveld	Naked Neck	Nondescript Village
Reporting year	-	-	2009	2020	-	-
Females	-	-	-	-	-	-
Males	-	-	-	-	-	-
Conservation site	-	-	SASBLIA	-	-	-

N.B. (to be note): SASBLIA—South African Stud Book and Livestock Improvement Association.

On the other hand, samples containing the genetic material of these indigenous chickens for genebank purposes have been conserved in vitro (Table 9). This is so that they may be used in future for reproducing, especially since the extinction status for most of these indigenous chickens is unknown, including nondescript village chickens. However, it is our opinion that in situ conservation of South African indigenous breeds continues to be a challenge due to limited knowledge of the significance of conservation in rural farmers and the importance of reporting the existing data if any [15]. For instance, there is no data reported on DAD-IS database with regards to anything related to nondescript village chickens and these data are also vital for researchers to be able to keep track of the existence of these chickens and further implement conservation strategies. To combat the issue of low reporting rates on the DAD-IS database, the Department of Agriculture should make it mandatory to improve reporting of the existing data, if any, and to update the records.

Table 9. Conservation of South African indigenous chicken breeds in vitro [21].

Population Data	Potchefstroom Koekoek	Venda	Ovambo	Boschveld	Naked Neck	Nondescript Village
Reporting year	2020	2019	-	-	2019	-
Semen samples frozen	-	0	-	-	0	-
Semen donors	-	0	-	-	0	-
DNA samples	-	0	-	-	0	-
DNA male donors	-	0	-	-	0	-
DNA female donors	-	0	-	-	0	-
Total DNA donors	-	0	-	-	0	-
Sample collectors	-	ARC	-	-	ARC	-
Breed extinction status	Unknown	Unknown	-	-	Unknown	-

Characterization of semen in South African indigenous chicken breeds, as observed in Table 10, was evaluated. A lot of research studies have been focusing on conducting groundwork on the analysis of the semen parameters or the characterization and improvement of indigenous chicken breeds like Potchefstroom Koekoek [19,25], Venda [18,26], and Ovambo [19]; however, the motility rate post-freezing in all of these studies is still below 50%. Moreover, it is our view that there is not much or no semen characterization performed on breeds like Boschveld, Naked Neck, and nondescript village chickens—for which their extinction status is unknown—with no characterization of semen parameters and conservation strategies such as cryopreservation.

Table 10. Semen characteristics of South African indigenous chicken breeds post-cryopreservation.

Chicken Breed	Semen Volume (mL)	Semen pH	Sperm Concentration ($\times 10^9$)	Progressive Motility (%)	Total Motility (%)	References
Potchefstroom Koekoek	0.3	7.6	-	-	3.0	[25]
	-	-	-	-	47	[19]
Venda	0.3	6.9	6.8	1.7	5.6	[26]
Ovambo	0.3	6.8	53.0	13.4	39.7	[18]
	-	-	-	-	45	[19]

9. Challenges During Conservation of South African Indigenous and Nondescript Village Chickens

9.1. Lack of Resources

In rural settlements, indigenous chickens are kept and raised under minimum-input free-range management conditions. Under such circumstances, it is likely that little to no attention is paid to housing, feeding, breeding, or controlling parasites, germs, and illnesses [53]. This can be partially explained by the uncontrolled breeding practices, which lead to an endless cycle of multiple matings of chickens released into the environment, as well as the variations in the availability of scavenging feed resources based on local climate conditions [33]. Moreover, in most cases, chickens will be walking around the households in search of food like insects, vegetables, seeds, and discarded leftovers [8]. When such behaviors persist and no proactive steps are taken to avert illnesses, significant financial losses typically result from the spread of infectious diseases. The diseases previously reported include fowl typhoid, Newcastle disease, fowl cholera, and infectious coryza, and most rural households cannot afford or do not have vaccination measures in place. According to the survey results by [54], indigenous chickens were susceptible to virulent NDV because of the lack of vaccination and biosecurity. Moreover, about 47% of the rural farmers are using traditional remedies for the prevention and treatment of diseases. Furthermore, Mtileni et al. [5] reported that Newcastle disease was the major cause of chicken loss (38.7%) and production. This is due to challenges in poor disease control and an absence of proper vaccination and extension services. As a result of these occurrences, the production rate of these chickens is now declining, yet differences in productive performance across various locations indicate that improvements may be possible without requiring significant adjustments to low-input systems [9]. This calls for an intensive introduction of community-based vaccination programs in all provinces that will help and guide farmers in order to combat the spread of diseases. For instance, in districts like Mpendle, there have been such programs that were introduced by the Department of Agriculture to assist and train farmers with vaccinations for diseases like Newcastle disease, and to this point, these programs have been progressing successfully. Moreover, there are vaccination programs led by the Department of Agriculture (Newcastle) that are currently running for species like cattle to curb the spread of diseases and, as such, can also be mandated for indigenous chickens as a tool to fight against diseases and improve conservation.

9.2. Lack of Knowledge/Education

The majority of rural farming communities comprise smallholder/subsistence farmers who are not just the backbone of agricultural-dependent economies, they are the lifeblood of the global food system in endeavors to secure poverty alleviation and zero hunger, as outlined in the Sustainable Development Goals. Moreover, these farmers contribute immensely to supplying local and international markets with the required food for homes, restaurants, and other businesses. Nevertheless, limited access to education has been an incurring challenge, delaying the ability for growth and innovation within these farming communities [55]. Most South African rural farmers keep indigenous animals with their traditional knowledge but have less knowledge of the importance or emphasis of those animals and their pride [56]. Some of them will be selling the most precious animals to auctions not knowing that they hold value not only to them but globally. Due to these considerations, it is more difficult to raise awareness among South African smallholder farmers of the global need for the conservation of farm-animal genetic resources (AnGR), which is currently inadequate [15]. It is even a challenge to brief smallholder farmers on the latest technologies because most of them are old farmers who do not hold qualifications or matric certificates, making it difficult for them to understand what you are saying in plain language. However, it seems that smallholder farmers' lack of access to quality education in rural locations may change their motivation and excitement towards valuing and learning more about agriculture [57]. Through social gatherings, farmers' study groups, meetings with village leaders, input suppliers, extension officials, and representatives of non-governmental organizations (NGOs), as well as agricultural exhibits, smallholder farmers exchange and obtain agricultural information [58]. For instance, South Africa's National Department of Agriculture established the Kaonafatso Ya Dikgomo (KYD) scheme led by the Agricultural Research Council (ARC) to assist smallholder cattle farmers, and a similar strategy should be employed for chicken rearers. To further assist in transferring knowledge to these farmers, training modules composed of topics that can easily instill skills and transfer knowledge to these farmers would be a good initiative. For instance, the Department of Agriculture in provinces such as Gauteng has introduced study group sessions where they invite experts from research institutes (such as the ARC) to come and teach farmers on requested modules for different species. It should be mandated that such training and sessions are initiated in all provinces by officials in collaboration with experts to help these farmers achieve a better understanding and farm sustainably. Furthermore, farmers should be introduced to the use of mobile apps to be able to communicate with experts and gain knowledge; for instance, the ARC has developed an app called the ARCHub app, with manuals in various disciplines that can be of use to farmers. In addition, it should be a formal mandate for these stakeholders to encourage young people to take part and show an interest in agriculture.

9.3. Frozen–Thawed Cockerel Semen Quality Hindering Application of Artificial Insemination

Semen cryopreservation can be described as the process of freezing male gametes at −196 °C. Numerous indigenous breeds lack characterization and face extinction as a result of evolving production practices that favor exotic commercial breeds and discourage crossbreeding [29]. There is a high mandate for semen cryopreservation technology and the preservation of germplasm resources where chickens are concerned [59]. This is attributed to the fact that chicken females have distinct physiological traits and, thus, it is challenging to preserve their oocytes or embryos [60]. Moreover, because sperm cell survival after the freeze–thaw procedure is poor, cryopreservation of cockerel semen continues to be a difficult challenge [18]. Furthermore, using frozen–thawed semen for AI results in an extremely low fertilization rate [17]. Even with advancements in the development of cryopreservation techniques and semen diluents, chicken semen survival and motility rates are still low [18]. Studies on molecules have demonstrated that one of the main reasons for low-quality post-thawed sperm is the damage to proteins, lipids, and ions during cryopreservation [61].

9.4. Outbreaks of Diseases

One crucial technology for the sustainable breeding of chicken species is the cryopreservation of their reproductive cells. Chicken farms suffer significant losses due to the epidemics of infectious avian diseases, especially the highly pathogenic influenza virus, which also leads to a significant death toll and requires the decontamination of affected premises [62]. Village chicken production does not follow the government's recommended biosecurity precautions, such as managing sick birds, which increases the risk of illness [34].

Therefore, in order to be ready for unexpected epidemic outbreaks, chicken breeds must be biobanked. Furthermore, it is mandated to preserve the genetic diversity of locally adapted indigenous chicken breeds, which are present globally [38], in order to stop their extinction. Additionally, new developments in genome editing technology enable us to effectively generate genetically modified chickens, including those that lay eggs with fewer allergens [63] or possess specific virus resistance, highlighting the significance of biobanking commercial and academic chicken lines [62].

9.5. Chicken Reproductive Physiology

Chickens are known to have one functioning ovary, unlike other mammals which have two functioning ovaries. The right ovary stops growing when the female chick hatches, while the left one continues to mature and, hence, only the left ovary remains functioning for the rest of the chicken's reproductive life. Moreover, as of right now, semen cryopreservation is the only non-invasive and less-expensive in vitro technique available for the ex situ in vitro conservation of chicken germplasm and the preservation of endangered breeds [64]. As a result, it has been difficult to cryopreserve chicken breeds because sperm collection and storage have been the only method available for conserving male genetic material [65]. Female genetic material cannot be cryopreserved because of the characteristics of the chicken egg [60]. This is a limiting factor because only some of the genetic diversity of the chicken breed can be preserved. Methods for cryopreserving gonadic tissue and primordial germ cells have been developed [66]; however, despite an increasing interest in these methods and ongoing advancements in their developments [67], these technologies remain extremely invasive and more pricey than semen procedures.

10. Future Projections or Resolutions to Enhance South African Indigenous Chicken Breeds

10.1. Semen Cryopreservation

Semen can be collected in cockerels via the abdominal massage method. After collection, semen should be qualified and quantified by analyzing for motility, morphology, concentration, and pH. Equipment like pricey microscopes, computers, and lab supplies are necessary for this type of analysis, and smallholder farmers lack the funds to hire research stations, some of which are located far away. Though semen cryopreservation is the only ART permitting the use of semen even after the male is dead, in chickens, only 39.5% remains viable and motile after freezing [18].

Semen extenders can be used as a medium for preserving and or conserving sperm to facilitate fertilization. Additionally, semen extenders can prevent cryogenic damage, manage bacterial transmission and contamination, maintain and preserve sperm metabolic activities, and regulate the pH of the medium during and after thawing [68]. As would be assumed, semen extenders also need to provide the sperm with other qualities, including energy, antioxidants to lessen oxidative stress, anti-freezing shock protection, and antibiotics to avoid contamination [69]. There are two types of extenders: a liquid form, which lasts for three days on average; and a cryopreserved form, which lasts for years [70]. Depending on the kind of sperm extender and species, several material sources can be used, e.g., animal sources, egg yolks, skim milk [71], and plant sources (soybean lecithin) [72]. Each of these sources offers distinctive characteristics and challenges.

10.2. Enhancement of the Reproductive Performance of Indigenous Chickens Through Breeding and Selection

For agriculture to remain sustainable, the complex methods involved in local chicken breeding and gene improvement are essential [8]. These processes depend on the breeds chosen for use and require in-depth understanding of each breed's distinctive traits or phenotypes [8]. Animal breeders use these methods to develop agricultural animals that are more resilient and productive. Moreover, food and animal sectors have also made use of ARTs, such as AI, semen collection, and preservation. Nevertheless, while considering genetic improvement in indigenous chickens, several elements need to be taken into account. For instance, integrating single genes that change or lower feathering, such as the naked-neck, scaleless, and frizzle genes, can improve endurance to high temperatures, which is a critical requirement [8]. Indigenous chickens can also be enhanced by the use of breeding techniques like within-line selection, back-crossing, and crossbreeding [73]. In order to improve the growth performance and body weight of indigenous chickens raised in an intensive management system, crossbreeding may be necessary [74]. However, little scientific data exist on Southern African chicken crossbreeding to improve growth qualities [75]. Additionally, the focus of chicken development in under-developed countries has been on exotic breeds [76]. Another thing that needs to be avoided at all costs is inbreeding, as it can lower the productivity of indigenous chickens [77]. However, there is minor information available regarding the genetic improvement of indigenous chickens in Southern Africa [8]. Therefore, comprehensive genetic improvement plans targeting indigenous chickens must be developed and put into practice.

10.3. Semen Quality Improvement Through Dietary Supplementation

Long-chain polyunsaturated fatty acid supplementation, especially of omega-3, benefits in improving fertility and semen quality [78]. This was employed as an approach for enhancing the production of high-quality sperm. Moreover, to keep the lipid bilayer stable, polyunsaturated fatty acids must be present in the cellular membrane [79], whereas the lipids of the sperm are there for the flexibility and active movement of the sperm. Nevertheless, if given in high concentrations, polyunsaturated fatty acids in the membrane causes the sperm to become extremely susceptible to ROS, which leads to lipid peroxidation. This then necessitates the supplementation of antioxidants to strike a balance between the production of ROS and available defense antioxidants like ascorbic acid. The main component of the sperm's antioxidant system works to prevent apoptosis and protect the membrane from reactive oxygen species (ROS) in order to maintain the integrity and functionality of sperm [80]. Hopcroft et al. [81] discovered that adding 50 g/kg of fish or corn oil to the diets of hens produced a significantly greater (96%) fertility rate than the rate that existed before the supplements (89%). Alagawany et al. [82] observed that increased ω-3 fatty acid proportion in sperm phospholipids resulted in better fertility at 39 weeks of age when male diets are supplemented with LCPUFAs. Cerolini et al. [83] revealed that supplementing dietary LCPUFAs can affect spermatozoa characteristics. Fish oil supplementation to cockerel diets increased fertility, which was explained by the sperms reduced fatty acid ratio (ω-3), which may alter the membrane's physical characteristics or resistance to peroxidative damage [84,85]. It has been discovered that cryopreservation affects both sperm quality and fertility and LCPUFAs protect sperm. Semen cryopreservation has an impact on survivability, which is mostly influenced by the lipid content of spermatozoa [84,85]. Additionally, after cryopreservation, Hudson et al. [84] observed a drop in the cholesterol/phospholipid ratio in cockerel sperm and a correlation with fluidity, which affected survivability. Long-chain polyunsaturated fatty acids can protect sperm from chemical (oxidative) and physical (cryopreservation) damage.

11. Conclusions

With reference to the Domestic Animal Diversity Information System, it is our view that there is little to no information and a lack of reporting (mandatory factor) on the population data of South African indigenous chicken breeds like Potchefstroom Koekoek, Ovambo, Boschveld chicken, and nondescript village chickens, necessitating interventions such as ARTs (e.g., AI), which are widely used in other species to curb low fertility rates and for efficient breeding purposes. It is also important to intensify and employ conservation strategies such as the in situ and ex situ conservation of gametes like semen where chickens are concerned since it is almost impossible to preserve their ovaries due to the reproductive physiological structure. It is to be noted that these strategies are all important in order to improve efficiency, mitigate the risk of extinction, and respond to the SDGs. Thus, it remains a mandate for policy makers as well as NGOs to partake in supporting and implementing the use of these strategies. Moreover, a lack of knowledge has been discovered as one of the factors that limit rural farmers from being able to produce, reproduce, and farm sustainably.

This can be avoided by an active response of the introduction of study groups, recognition of prior learning training, and certification to allow farmers to participate in a broader spectrum and gain skills; information days and farmers days, hosted by the Department of Agriculture in conjunction with institutions like research stations and universities; and schemes like KYD, the same as is practiced with small livestock and cattle. As mentioned previously, in some provinces, the Department of Agriculture has already introduced study sessions and training where experts on the subjects are invited to give lessons on selected farming modules. Now, to effectively respond to poverty alleviation and Zero Hunger goals, it is important for all provinces to partake in the same initiative to help farmers with production and reproduction practices to reach the market. However, it is to be noted that the success of these initiatives depends on the joint collaboration between the Department of Agriculture supported by stakeholders like research institutes, NGOs, and policy makers.

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