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Sustainable and Innovative Practices in Animal Breeding and Nutrition

Edited by Yusuf Bozkurt and Mustafa Numan Bucak



Sustainable and Innovative Practices in Animal Breeding and Nutrition

*Edited by Yusuf Bozkurt
and Mustafa Numan Bucak*

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Sustainable and Innovative Practices in Animal Breeding and Nutrition

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IntechOpen Book Series

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Aims and Scope of the Series

Paralleling similar advances in the medical field, astounding advances occurred in Veterinary Medicine and Science in recent decades. These advances have helped foster better support for animal health, more humane animal production, and a better understanding of the physiology of endangered species to improve the assisted reproductive technologies or the pathogenesis of certain diseases, where animals can be used as models for human diseases (like cancer, degenerative diseases or fertility), and even as a guarantee of public health. Bridging Human, Animal, and Environmental health, the holistic and integrative “One Health” concept intimately associates the developments within those fields, projecting its advancements into practice. This book series aims to tackle various animal-related medicine and sciences fields, providing thematic volumes consisting of high-quality significant research directed to researchers and postgraduates. It aims to give us a glimpse into the new accomplishments in the Veterinary Medicine and Science field. By addressing hot topics in veterinary sciences, we aim to gather authoritative texts within each issue of this series, providing in-depth overviews and analysis for graduates, academics, and practitioners and foreseeing a deeper understanding of the subject. Forthcoming texts, written and edited by experienced researchers from both industry and academia, will also discuss scientific challenges faced today in Veterinary Medicine and Science. In brief, we hope that books in this series will provide accessible references for those interested or working in this field and encourage learning in a range of different topics.

Meet the Series Editor



With more than 35 years of academic experience, Dr. Rita Payan-Carreira is now a Full Professor at the University of Évora (UE). She specializes in animal theriogenology, with a particular focus on the determinants of female fertility and fetal-maternal interaction during implantation. Dr. Payan-Carreira is dedicated to promoting a culture of collaboration and knowledge-sharing within the scientific community. In addition to her extensive publication record, she frequently collaborates with various publishing houses on peer-review activities. She further contributes to the scientific advancement of students and professionals by supervising academic theses and offering technical and scientific courses, including scientific communication, critical reasoning and discussion, and the ethical aspects of research.

Meet the Volume Editors



Yusuf Bozkurt received his B. Sc., M. Sc., and Ph.D. degrees from Ankara University, graduating with an Honors Certificate. He is currently a Professor of Reproductive Biotechnology in Aquaculture at Mersin University. He has enhanced his academic expertise through research visits and collaborations at several international universities and institutions. His work focuses on reproductive biology, biotechnology, and the conservation of aquatic genetic resources.



Mustafa Numan Bucak received a bachelor's degree from Ankara University Veterinary Faculty and a Ph.D. in sperm cryobiology from Ankara University. He serves as an academic staff member of the Department of Reproduction and Artificial Insemination at Selçuk University, Veterinary Faculty. He manages several studies on sperm and embryos. He serves on the editorial boards of international journals. His studies include sperm cryobiology, in vitro fertilization, and embryo production in animals.

Contents

Preface	XIII
Section 1	
Cryobiology and Ex-Situ Conservation Technologies	1
Chapter 1	3
The Crucial Role of Cryopreservation in <i>ex-situ</i> Conservation of Caspian Sea Sturgeons <i>by Yusuf Bozkurt and Mohammad Hassanzadeh Saber</i>	
Chapter 2	21
Current Advances in Improving the Quality of Bovine Semen After Thawing Through the Addition of Different Antioxidants <i>by Ana Prada, Maria Helena Moreira da Silva and Fernando Moreira da Silva</i>	
Section 2	
Reproductive Biotechnologies in Animal Production Systems	33
Chapter 3	35
The Contribution of Reproductive Technologies to Dairy Cattle Genetic Improvement in the Amhara Region: Successes and Challenges in Research and Development Efforts <i>by Assemu Tesfa, Birhan Kassa, Mengistie Taye, Ahemed Alkader, Demelash Aychilie, Zemenu Birhan, Tarekegn Demeke and Mesfin Lakew</i>	
Section 3	
Small Ruminant Production and Rural Livelihoods	57
Chapter 4	59
Husbandry Practices, Performance Potentials, Breeding Strategies and Socioeconomic Roles of Black Bengal Goat to Enhance the Food Security, Empower Women and Improve the Rural Livelihoods in Bangladesh <i>by Auvijit Saha Apu, Md. Tayef Arefin, Israt Jahan, Mohammad Mahbubul and Younus Ali</i>	
Section 4	
Genetic and Phenotypic Innovations in Fiber Livestock	85

Chapter 5	87
New Insights into the Color Phenotypes and Genotypes of Huacaya Alpaca and Their Fiber's Economic and Commercial Value <i>by Rubén Pinares</i>	
Section 5	
Alternative Feed Resources and Sustainable Poultry Production	103
Chapter 6	105
The Potential Role of Azolla as an Alternative Protein Source in Sustainable Poultry Farming: A SWOT Analysis <i>by Soumaya Boukrouh</i>	

Preface

The global demand for animal-derived food, fiber, and income is increasing, while biodiversity conservation, resource efficiency, and sustainable livelihoods are becoming more urgent. Population growth, environmental degradation, and climate change intensify these challenges, making innovations in animal reproduction, genetics, and feeding systems essential for sustainable production and conservation.

This book, “Sustainable and Innovative Practices in Animal Breeding and Nutrition”, brings together studies that aim to make animal production systems more resilient, inclusive, and environmentally responsible. It covers both aquatic and terrestrial species and shows how reproductive biotechnologies, genetic improvement, and new feed resources can contribute to food security and biodiversity conservation.

Section I, *Cryobiology and Ex-Situ Conservation Technologies*, highlights the importance of cryopreservation in protecting genetic resources.

The first chapter, “The Crucial Role of Cryopreservation in Conservation of Caspian Sea Sturgeons”, explains how gamete and embryo freezing support the conservation of endangered sturgeons by helping maintain genetic diversity. The second chapter, “Current Advances in Improving the Quality of Bovine Semen after Thawing Through the Addition of Different Antioxidants”, discusses the challenges of cryopreservation in livestock and presents recent progress in reducing oxidative stress to improve post-thaw semen quality and fertility.

Section II, *Reproductive Biotechnologies in Animal Production Systems*, explores how reproductive technologies are used to improve productivity and genetic progress, especially in smallholder and regional settings. The chapter “The Contribution of Reproductive Technologies to Dairy Cattle Genetic Improvement in the Amhara Region: Successes and Challenges in Research and Development Efforts” provides a detailed case study from Ethiopia, examining how reproductive technologies and institutional support have shaped genetic improvement in dairy cattle.

Section III, *Small Ruminant Production and Rural Livelihoods*, highlights the vital contribution of small ruminants to household food security, gender empowerment, and rural development. The chapter “Husbandry Practices, Performance Potentials, Breeding Strategies and Socioeconomic Roles of Black Bengal Goat to Enhance the Food Security, Empower Women and Improve the Rural Livelihoods in Bangladesh” provides a comprehensive account of how indigenous goat breeds serve as both economic assets and instruments of empowerment, particularly for rural women.

Section IV, *Genetic and Phenotypic Innovations in Fiber Livestock*, focuses on advances in animal genetics and value-added fiber production. The chapter “New Insights into the Color Phenotypes and Genotypes of Huacaya Alpaca and Their Fiber’s Economic and Commercial Value” examines the molecular and economic aspects of alpaca fiber

color, showing how genetic variation contributes to market diversification and supports sustainable textile development.

Finally, Section V, *Alternative Feed Resources and Sustainable Poultry Production*, focuses on one of the key challenges in livestock systems—developing sustainable feeding strategies. The chapter “The Potential Role of Azolla as an Alternative Protein Source in Sustainable Poultry Farming: A SWOT Analysis” evaluates Azolla as a low-cost, high-protein feed option, examining its nutritional value, environmental benefits, and socioeconomic implications, along with its strengths, weaknesses, opportunities, and threats for use in smallholder poultry production.

These contributions collectively illustrate the synergy between reproductive efficiency, genetic preservation, and feeding practices in promoting sustainable and innovative animal production. Ranging from the conservation of endangered sturgeons and the genetic improvement of dairy cattle to women’s empowerment through goat farming and new feed resources for poultry, the chapters present a holistic view of sustainability that connects scientific research, practical application, and policy.

We hope this book will serve as a valuable resource for researchers, educators, policymakers, and students in animal science, veterinary medicine, and agricultural development. By combining laboratory research with field practices, this book promotes interdisciplinary knowledge sharing and fosters innovative approaches to sustainable animal production and genetic resource conservation. We sincerely thank all the authors for their valuable contributions and express our gratitude to Ms. Mirna Papuga, Publishing Process Manager at IntechOpen, for her support throughout the publication process.

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Section 1

Cryobiology and Ex-Situ Conservation Technologies

Chapter 1

The Crucial Role of Cryopreservation in *ex-situ* Conservation of Caspian Sea Sturgeons

Yusuf Bozkurt and Mohammad Hassanzadeh Saber

Abstract

The Caspian Sea holds the reputation of being the largest enclosed water reservoir in the world. Despite the weaker ichthyofauna found in the Caspian Sea, the productivity of industrial fishing has remained consistently high. For centuries, the Caspian Sea has been a key location for sturgeon production on a global scale. However, the number of sturgeons in the Caspian Sea is dwindling and facing significant dangers caused by some factors including environmental degradation, the building of dams, reduced water flow, intense fishing pressure, water pollution, and these factors damage sturgeon's natural spawning areas. Hence, the Caspian Littoral States must take significant scientific steps to successfully organize both regional and national efforts to create a timely action plan to safeguard sturgeon populations. Through this review, we aim to describe the significance of *ex-situ* conservation strategies in safeguarding the endangered Caspian sturgeon species, with a focus on the use of cryopreservation.

Keywords: Caspian sea, sturgeon, *ex-situ* conservation, cryostorage, frozen gene banks

1. Introduction

Sturgeons, members of the *Acipenseridae* and *Polyodontidae* families, are considered one of the primary freshwater fish species of utmost importance in aquaculture on a global scale, owing to their delectable black caviar, meat, and isinglass. Sturgeons are considered primitive vertebrates worldwide and are often referred to as “living fossils.” It is believed that they have resided in the northern hemisphere water for 200 million years [1].

The largest brackish water lake globally, the Caspian Sea, is home to six different species of sturgeon. The Caspian Sea has long been recognized as the sturgeon sea due to its contribution of over 90% of the global caviar supply [2]. However, their natural population is now highly endangered due to overfishing for delicate flesh and black caviar, pollution of freshwater, destruction of spawning habitats, and barriers

created by humans to spawning migration. All sturgeon species have been officially declared as threatened with extinction, as stated by the Red List published by the International Union for Conservation of Nature [3].

It is presently evident that traditional measures, such as reducing pollution and preserving habitats, have not been successful in maintaining and restoring sturgeon populations. Currently, living sturgeon populations are at risk and require stock enhancement and sustainable conservation programs for survival [1]. Therefore, it is crucial to incorporate biotechnological techniques swiftly into *ex-situ* conservation schemes.

The preservation of genetic material of aquatic species through cryopreservation and the establishment of gene banks is highly significant for managing fisheries and enhancing the cultured production of endangered sturgeons in the Caspian Sea. In light of the potential extinction of sturgeon in the Caspian Sea, it is crucial to preserve their sperm through *ex-situ* methods to ensure their long-term conservation. Thus, biotechnological tools such as cryopreservation should promptly be integrated into *ex-situ* conservation initiatives such as cryobanking [2].

From this point of view, this review aims to discuss the significance of *ex-situ* conservation strategy for the conservation of the endangered Caspian sturgeon species, focusing on cryopreservation biotechnology.

2. Bio-ecological features of sturgeons

In spite of being considered to be among the primitive groups of Vertebrates, they have successfully adapted to current environmental conditions and hold distinct advantages over Teleost fishes. These advantages can be summarized as a broad selection of ideal spawning temperatures, quick acclimation to different levels of salinity in juvenile fish, and a varied range of feeding options.

While some species reside in freshwater their entire lives, others embark on a journey towards brackish or seawater environments once they reach a certain size, often achieving significant lengths of up to 6–7 meters [4]. Sturgeons spend their growing period in the deep waters of the sea, but reproduce in rivers. The reproductive migration of adult individuals from sea to freshwater varies depending on the temperature of their environment, but generally begins in the spring when the water warms up.

Sturgeons reach sexual maturity and begin spawning only after reaching the age of 7–12 years. The sturgeons spawn in the Caspian basin during the period between mid-May and early June, and the water temperature typically falls within the range of 8 to 14°C [5]. Spawning occurs at 2–10 m depth of rivers, in places with fast currents and gravel bottoms, and the quantity of eggs released by a female sturgeon can vary from 100,000 to 3 million. Sticky eggs, which are 2–4 mm in diameter and vary in color from dark-gray to black, depending on the species, cling to the pebbles and stones at the bottom. After laying its eggs in the oxygen-rich, gravelly waters of the rivers, the migration is completed when it returns to the deep waters of the seas [6].

Fly larvae, worms, various molluscs, and crustaceans predominate in the diet of sturgeons. They usually reveal these foods by mixing the soft soles with their long nose and suck them with their proboscis-shaped mouth. In addition to these foods, large sturgeons also eat small fish [6].

Sturgeons possess a cartilaginous skeleton, a feature that distinguishes them from bony fish species that exist today. The notochord has a protective covering called the

perichord, which provides structural support for the cartilage [7]. The spinal cord is situated superior to the notochord. Typically, the caudal fin displays a heterocercal design, allowing the spinal cord to continue into the upper body. Sturgeons possess a coiled intestinal valve that enhances the nutrient absorption area and prolongs food digestion duration. The swimbladder is basic and physostomous, linking directly to the digestive system. The body is protected by five lines of bony dermal plates [5].

Sturgeons have a strong ability to hybridize, and studies have shown that hybridization is a widespread occurrence in nature, involving nearly all species. However, when sturgeons from the same species or different species mate ($2n \times 2n$ or $4n \times 4n$), offspring from individuals with identical ploidy levels are fertile, but hybrids from differing ploidy levels ($2n \times 4n$) are typically sterile, with a chromosome count of roughly 160–180. This is crucial since if hybrids are used extensively in sturgeon farming, there's a risk of them escaping into the wild and genetically contaminating natural sturgeon populations in waterbodies [5].

3. Home of sturgeons: The Caspian Sea – its features and sturgeons

The largest body of water situated inland worldwide is the Caspian Sea. Although classified as a lake in geographical terms, this water body is commonly referred to as a sea due to its many hydrological characteristics [8].

The Caspian Sea holds significant historical value and exhibits various distinct geopolitical, environmental, and economic characteristics. The Caspian Sea's boundary includes Azerbaijan, Russia, Iran, Turkmenistan, and Kazakhstan, while there are a total of eight countries in the Caspian Sea region, including Türkiye, Azerbaijan, Kazakhstan, Turkmenistan, Russia, Iran, Georgia, and Armenia (**Figure 1**). It is crucial to be aware that the Caspian Sea is impacted by various ecological factors due to the rivers that flow through the territories of the states located in the Caspian basin [9].

The maximum depth recorded in the Caspian Sea is 1025 meters, and a total coastline length of 7010 kilometers [11]. The average depth of the Caspian Sea's northern section is only 4 to 6 meters, making it quite shallow. The southern section, encompassing one-third of the entire area, has a maximum depth of 1000 meters. The sea's middle region is mainly a shelf expanse, with depths that can reach up to 100–150 meters [12].

The riverine-marine ecosystems of the Caspian Sea, characterized by warm water temperatures, acidic water, and the inflow of numerous rivers, provide an optimal environment for a diverse range of fish species [13]. In addition, the Caspian Sea is extremely important due to its valuable fish resources. Although the fish community residing in the Caspian Sea is not as strong as in other seas in terms of large-scale fishing, it has always been highly productive. The Caspian Sea was rich in roach, kille, herring, bream, pikeperch, common carp, perch, and other fish species, with sturgeons being the most prized catch, amounting to thousands of tons.

Compared to other areas around the world, the Caspian Sea Basin is renowned for its extensive assortment of sturgeon species. The Caspian Sea has long been known as the primary source of sturgeon, producing over 90% of the world's caviar. Despite a significant decline in the sturgeon population in recent years, the Caspian Sea remains a critical area for sturgeon fisheries and aquaculture.



Figure 1.
The Caspian Sea and its immediate surroundings [10].

Beluga (*Huso huso*), ship (*Acipenser nudiiventris*), Persian sturgeon (*Acipenser persicus*), stellate (*Acipenser stellatus*), Russian sturgeon (*Acipenser gueldenstadti*), and sterlet (*Acipenser ruthenus*) are among the six sturgeon species found in the Caspian Sea, under the genera *Huso* and *Acipenser*. Several species are widespread in certain regions of the basin, while others are close to the point of extinction [14].

The mature female sturgeon's eggs are used to create black caviar, an opulent food item that comes with a hefty price tag. The majority of caviar produced by countries that are adjacent to the Caspian Sea (Iran, Kazakhstan, Russia, Turkmenistan, Azerbaijan) is derived from four specific species: Persian sturgeon (*A. persicus*), beluga sturgeon (*Huso huso*), stellate sturgeon (*A. stellatus*), and Russian sturgeon (*A. gueldenstaedti*). These types of fish are responsible for the production of osetra, beluga, sevruga, and Persian caviars, known by their respective trade names. While sturgeon stocks have decreased in

the last two decades, Russian, beluga, and stellate sturgeons play a critical role in the production of meat and caviar [15, 16].

4. Conservation status of Caspian Sea sturgeons

The Caspian Fisheries Research Institute has been carrying out monitoring of the population of sturgeon in the Caspian Sea for more than five decades, now with the active involvement of Azerbaijan and Kazakhstan. The number of sturgeons inhabiting the waters of the Caspian Sea, including those used for commercial purposes and those that enter spawning locations in the Volga River, has been on a steady decline for many years [17].

Furthermore, the continued existence of the sturgeon's future generation is facing significant threats. The degradation of the environment, including the erection of dams, restricted water flow, high fishing pressure, and water pollution, caused a decrease in the sturgeon stocks owing to their inability to migrate and breed in these conditions [18]. Additionally, the destruction of the sturgeon's natural spawning habitats, the development of unfavorable wintering conditions beneath the dam, the loss of breeding grounds and water quality, and the gradual decline in the amount of mature sturgeon are other reasons for the extinction of the sturgeon species existing in the Caspian Sea. In the absence of major improvements, the current unsatisfactory situation of the sturgeon resources will result in the extinction of the majority of sturgeon species in the Caspian Sea soon. Thus, how to safeguard the genetic resources of Caspian sturgeon has emerged as a key question in light of these issues [19].

Therefore, it seemed that it was imperative for the Caspian Littoral States to implement rigorous scientific protocols in order to effectively organize efforts at both regional and national levels towards the development of a prompt Action Plan for the protection of sturgeon stocks. For that reason, the necessity of creating the Commission on Biological Resources of the Caspian Sea arose from the need to develop a plan for the prudent use of common resources and to carry out a joint conservation program for this valuable asset. The CITES (The Convention on International Trade in Endangered Species of Wild Fauna and Flora) officially added all sturgeon species to its list in 1997, with implementation beginning in April 1998. Since that time, a number of joint efforts have been established to assess sturgeon populations, set quotas for sturgeon harvesting and export, and implement joint programs for the protection and administration of sturgeon resources. Concurrently, various global entities, including the UNDP, FAO, World Bank, and the European Commission (EU/TACIS), have been collaborating to design a regional program aimed at enhancing the current resource situation [20].

In this context, most Caspian states decided to begin construction of sturgeon hatcheries recently. The creation of numerous sturgeon hatcheries was a means of compensating for the disruption of areas crucial for fish spawning because of dam construction, particularly in the former Soviet Union and the Islamic Republic of Iran. As a result of these initiatives, the artificial propagation of sturgeon species and fingerling release was started in the Caspian states. Sturgeon fingerling production and distribution were maintained through the creation of 11 sturgeon hatchery centers in the USSR, as well as three and five

hatchery centers in Azerbaijan and Iran, respectively. In this sense, sturgeon populations in the Caspian Sea were significantly boosted by the crucial contribution of hatcheries [21].

The idea of rearing sturgeons in captivity has been proposed as an effective approach to their conservation and management [22, 23]. Conversely, it has been noted that raising sturgeon and paddlefish in captivity for commercial purposes does not serve as a solution to overfishing or as an efficient method for preserving these species in their original environments [24]. However, across the world, many conservationists consider stock enhancement and captive breeding as the only viable option for ensuring the sturgeon's survival [22].

Thanks to all these encouraging changes, there has been increasing interest in reducing the impact on native sturgeon populations by transferring commercial production to aquaculture in recent years [25]. Currently, sturgeon aquaculture has been focused on enhancing and conserving wild populations via controlled breeding production and also to produce farmed sturgeon broodstocks for caviar production [26].

5. Importance of *ex-situ* conservation programs for Caspian Sea sturgeons

Due to the reduction of sturgeon species in natural waters, there is an increasing emphasis on aquaculture methods as a viable and sustainable approach to fulfill the requirement for these species [27]. In this context, the aquaculture industry has been greatly influenced by advancements in biotechnology recently. There is a growing focus on lowering the expenses related to obtaining genetic data for individuals possessing favorable characteristics post-mating.

Maintaining the genetic diversity of sturgeon is key to their long-term survival and preventing them from becoming threatened in nature. Also, urgent actions must be implemented to protect the genetic variability of this species. Various conservation methods exist for endangered sturgeon species; however, ensuring the lasting integrity of their genetic makeup is crucial.

In this regard, it is clear that the implementation of *ex-situ* conservation, which involves “preserving biological diversity components away from their natural environments,” is inevitable. Captive broodstock can be maintained in specialized facilities like aquaria or aquafarms, while genetic material can be stored in frozen form in gene banks.

In recent years, there has been a rising level of concern surrounding *ex-situ* conservation programs on an international level. In numerous nations, *ex-situ* conservation is a key feature of complete conservation programs [28]. The primary goal of *ex-situ* conservation is to uphold the genetic makeup found in the original population source.

For the purpose of supporting aquaculture production and implementing successful recovery/conservation plans, the cryopreservation of sturgeon sperm is being regarded as a valuable strategy. It is important to highlight that cryopreservation is connected to cryobiology, which involves the storage of biological samples at extremely low temperatures, such as -196°C . The preservation of cellular viability is achievable by maintaining genetic stability at this specific low temperature [29, 30].

The underlying purposes of researching cryopreservation of fish sperm are to minimize costs related to live male brood stock, conserve threatened species, and make the most of individuals with excellent gamete quality for fertilization throughout the year. In addition, despite the existence of studies on freezing different fish reproductive cells, the most feasible means of protecting endangered fish species is through the cryopreservation of sperm.

Furthermore, by utilizing cryopreservation technology, restrictions concerning time and space in genetic breeding can be circumvented, making broodstock management more straightforward [19]. In this context, by utilizing sperm cryopreservation technology, it is possible to address the limitations of space and time on genetic information, thereby simplifying the conservation and distribution of gene sources with outstanding features [31].

In this context, cryopreservation technology allows for the storage of genetic materials like sperm, eggs, and embryos for future utilization in activities such as replenishing populations or serving as a foundation for breeding programs [28]. Among the genetic materials cryopreserved, mainly sperm cells are the preferred choice for genetic resource banking and genetic management programs for endangered species [32].

It is crucial to establish a collaborative regional initiative for freezing and storing sturgeon sperm, as well as facilitating its transfer among hatcheries, due to the shared sturgeon resources among the five Caspian Sea bordering countries. Hatcheries can participate in a regional program to exchange their knowledge and accomplishments, while also accessing the expertise of international institutions and scientific centers [14].

In this regard, it is imperative to create a detailed strategic regional plan that is interconnected and aligned with the national strategies of the Caspian Sea countries. It is essential for the end users to clearly establish the purpose of this particular plan. For instance, it is critical to determine the specific purpose for which the cryopreserved sperm will be used, whether it is utilized to replenish natural populations, support fish farming, or conduct a comprehensive preservation effort through storing sperm genes for various conservation goals [33].

From this point of view, cryopreservation biotechnology is vital in the *ex-situ* conservation of genetic material to preserve diversity. Hence, the adoption of cryopreserved semen can play a role in conservation efforts for the vulnerable Caspian sturgeon species by promoting stock enhancement [34]. As a result, cryopreservation of sperm from these endangered species will aid in hatchery efforts and research on the conservation of natural populations.

6. Features of sturgeon sperm and its cryopreservation

Sturgeon sperm possess certain conservative traits that teleosts lack as they evolved, and understanding these is key to successful cryopreservation. In comparison to teleost sperm cells, paddlefish and sturgeon spermatozoa have a longer cylindrical head, measuring around 5–6 mm, that is shielded by an acrosome filled with enzymes such as arylsulfatase and acrosine required for the acrosome reaction to occur in fertilization.

The acrosome reaction includes the development of a fertilization filament, which is suggested to function as a communicator informing the egg of a fertilizing sperm's

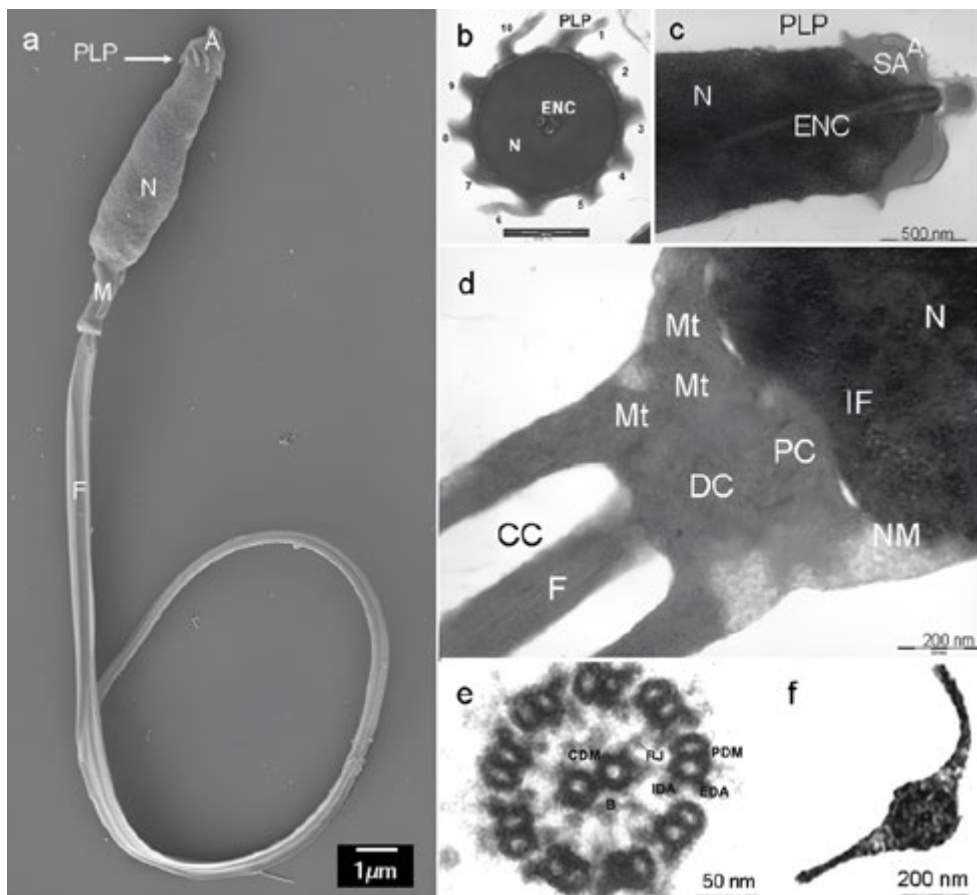


Figure 2.

Ultrastructure and morphology of different sturgeon sperm. Microscopic images are selected from research conducted on the Russian sturgeon (a): [37], Siberian sturgeon b, e: [38] and Persian sturgeon (c, d, F): [39]. A: acrosome, B: bridge, CC: cytoplasmic channel, CDM: Central Doublets Of Microtubules, DC: Distal Centriole, EDA: Outer Dynein Arms, ENC: Endonuclear Canals, F: Flagellum, IDA: Inner dynein arms, IF: Implantation Fossa, M: Midpiece, MT: Mitochondria, N: Nucleus, NM: Nuclear Membrane, PC: Proximal Centriole, PDM: peripheral doublets of microtubules, PLP: Posterolateral Projections, RJ: Radial Joins, SA: Subacrosome [36].

presence [35]. The sperm of sturgeon is categorized as a primitive type called an “aquasperm,” featuring an acrosome, head, midpiece, and flagellum organized with a microtubular structure of “9 + 2 (Figure 2). In all sturgeon species, the head of the sperm contains a nucleus with electron-dense chromatin and is elongated, enclosed by a nuclear membrane (Figure 2a, c, d). The acrosome, along with its sub-acrosomal regions, can be found situated at the apex of the sperm head (Figure 2a, c) [36].

Sturgeons have a seminal plasma with a low osmolality of 30–80 mosmol/kg, unlike teleost fish whose osmolality is around 300 mosmol/kg, even though their blood plasma is near 300 mosmol/kg. Sturgeons are known to have a large quantity of sperm, with larger males capable of producing up to 1–1.5 liters. However, the concentration of sperm is lower than that of teleosts, typically observed within the range of $0.5\text{--}2 \times 10^9$ spermatozoa/ml. The motility of activated sperm cells in sturgeons lasts for 5–6 minutes, a significantly longer period compared to teleosts, where it ranges from 20 seconds to 2 minutes.

The traits discussed earlier were key factors in determining the outcomes of development of a protocol for freezing sperm in sturgeons. The primary documented trials on cryopreservation of sturgeon sperm were conducted by Soviet researchers during the 1960s [40]. Up until the late 1990s, there were several publications discussing sperm cryopreservation in sturgeon [41–44]. The majority of the methods and outcomes that were documented could not be replicated or led to low rates of fertilization. The initial successful outcomes of sturgeon sperm cryopreservation were documented by 45.

Since then, many studies have been carried out on cryopreservation of sperm in different sturgeon species, including *Acipenser baeri* (Siberian sturgeon), *Acipenser fulvescens* (Lake sturgeon), *Acipenser gueldenstaedtii* (Russian sturgeon), *Acipenser persicus* (Persian sturgeon), *Acipenser sinensis* (Chinese sturgeon), *Acipenser stellatus* (Stellate sturgeon), *Acipenser sturio* (Atlantic sturgeon), *Acipenser transmontanus* (White sturgeon), and *Polyodon spathula* (Paddlefish) [46].

As illustrated in **Table 1**, these investigations have emphasized the importance of selecting the optimal cryoprotectant, extender, and their combinations, freezing and thawing rates [47–49].

7. Sperm cryobanks

The cryoconservation in biotechnology has become widespread, serving as a regular practice in aquaculture for tasks like selective breeding and fish hybridization. Furthermore, it plays a vital role in initiatives for preserving biodiversity and safeguarding endangered species. At present, cryogenic sperm banks are being established to safeguard endangered or uncommon species using cryopreservation biotechnology [60, 61].

This biotechnology makes it possible to conserve sperm samples for many species, and cryogenic sperm banks offer protection from unexpected circumstances or results [34]. It is essential to develop cryobanks for specific breeding stocks to initiate genetic selection initiatives in the aquaculture industry. It is also crucial for protecting endangered aquatic species until the environment improves [62].

Furthermore, the practice of sperm cryobanking offers various important advantages in terms of cost reduction, security enhancement, and workload management [63]. In addition, the management and shipping of frozen samples are relatively easy, allowing for flexibility in the planning of recovery programs. The cost of maintaining cryogenic gene banks is more economical than that of live gene banks.

According to the best of our knowledge, a trustworthy method for preserving cells through freezing of some sturgeon sperm has been developed, and several sperm cryobanks were established in some countries neighboring the Caspian Sea, such as Russia and Iran, for the aim of conservation and aquaculture [64–66].

8. Conclusion

In the face of the alarming decline of sturgeon populations in their natural environment, the steady growth of sturgeon aquaculture is highly critical. In this

Species	Extenders	Cryoprotectants	Freezing Rates	Thawing Rates	References
	30 mM Tris, 23.4 mM sucrose, 0.25 mM KCl, pH: 8.0	DMSO (10%) Methanol (10%)	4 cm above liquid nitrogen for 3 min, and then plunged into the liquid nitrogen	40°C/15s	[50]
<i>Huso huso</i>	23.4 mM Sucrose, 0.25 mM KCl, 30 mM Tris-HCl, pH: 8.0	Methanol (10%)	3 cm above liquid nitrogen for 3 min, and then plunged into the liquid nitrogen	40°C/5s	[51]
	0.2 M Maltose, 0.2 M Trehalose, 0.2 M Lactose, 0.2 M Lactulose	Methanol (9%)	3 cm above liquid nitrogen for 10 min, and then plunged into the liquid nitrogen	40°C/4s	[52]
<i>Acipenser persicus</i>	23.4 mM Sucrose, 118 mM Tris, 500 IU/ml penicillin potassium, pH: 8.0	DMSO (15%), Egg Yolk (20%)	3°C/min 20°C/min 25°C/min	40°C/25s 40°C/25s 40°C/25s	[53]
	23.4 mM Sucrose, 0.25 mM KCl, 30 mM Tris-HCl, pH:8.0	Methanol (10%)	-10°C/min -15°C/min -20°C/min -30°C/min -40°C/min	40°C/6s 40°C/6s 40°C/6s 40°C/6s 40°C/6s	[54]
	23.4 mM sucrose, 0.25 mM KCl, 30 mM Tris, pH8.0	MeOH (5%, 10%, 15%, 20%) DMSO (5%, 10%, 15%, 20%) Gly (5%, 10%, 15%, 20%) EG (5%, 10%, 15%, 20%)	3 cm above the liquid nitrogen for 10 min, and then plunged into the liquid nitrogen	37°C/10s	[55]
	0.2 M Maltose, 0.2 M Trehalose, 0.2 M Lactose, 0.2 M Lactulose	9% Methanol	3 cm above liquid nitrogen for 10 min, and then plunged into the liquid nitrogen	40°C/4s	[52]
<i>Acipenser gueldenstaedtii</i>	50 mM NaCl, 5 mM KCl, 10 mM Tris	10% DMSO	3 cm above liquid nitrogen for 10 min, and then plunged into the liquid nitrogen	35°C/10s	[56]
	30 mM Sucrose, 1 mM KCl, 25 mM, Tris	10% Methanol	3 cm above liquid nitrogen for 10 min, and then plunged into the liquid nitrogen	35°C/10s	[56]

Species	Extenders	Cryoprotectants	Freezing Rates	Thawing Rates	References
<i>Acipenser ruthenus</i>	23.4 mM Sucrose, 118 mM Tris, pH: 8.0	DMSO (15%), Egg Yolk (20%)	3.5°C/min 18.5°C/min	40°C/25s 40°C/25s	[57]
	23.4 mM Sucrose, 0.25 mM KCl, 30 mM Tris-HCl, pH: 8.0	Methanol (10%)	3 cm above the liquid nitrogen for 10 min, and then plunged into the liquid nitrogen	40°C/6s	[45]
	23.4 mM Sucrose, 0.25 mM KCl, 30 mM Tris-HCl, pH 8.0	Methanol (10%)	3 cm above the liquid nitrogen for 10 min, and then plunged into the liquid nitrogen	40°C/6s	[58]
<i>Acipenser stellatus</i>	23.4 mM Sucrose, 118 mM Tris, 500 IU/ml penicillin potassium, pH: 8.0	DMSO (15%), Egg Yolk (20%)	3°C/min 20°C/min 25°C/min	40°C/25s 40°C/25s 40°C/25s	[53]
	23.4 mM Sucrose, 0.25 mM KCl, 30 mM Tris pH: 8.0	DMSO (5%, 10%, 20%)	3 cm above the liquid nitrogen for 3 min, and then plunged into the liquid nitrogen	40°C/5s	[59]

Table 1.
 Important parameters in cryopreservation of caspian sturgeon species.

regard, sturgeon aquaculture should focus on controlled propagation, aiming to enhance and restore the natural populations.

The implementation of induced spawning and husbandry techniques has paved the way for restoration initiatives for sturgeons and paddlefish, and has also enabled the cultivation of these fish through farming. Consequently, there is a need for further development and optimization of sperm cryopreservation protocols to ensure the provision of viable sperm for activities such as population recovery, hybridization, conservation of endangered chondrostei species, sex manipulation, including androgenesis and gynogenesis, and also improvement of the genetic quality of broodstock.

Furthermore, establishment of the gene pools is crucial because the Caspian Sea sturgeon species is threatened by many reasons. Thus, the scientific efforts regarding the conservation of the endangered Caspian Sea sturgeon species must prioritize preservation of their genetic structure. In light of this context, *ex-situ* conservation programs are expected to achieve success in attaining the goal with the help of cryopreservation biotechnology.

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Current Advances in Improving the Quality of Bovine Semen After Thawing Through the Addition of Different Antioxidants

Ana Prada, Maria Helena Moreira da Silva and Fernando Moreira da Silva

Abstract

Oxidative stress has a negative impact on semen quality. Sperm handling and storage raise the probability of oxidative stress in cattle production. Therefore, there is a need to develop methods that can optimize sperm quality, especially when it is cryopreserved. The objective of this article was to analyze existing information on the current advances for the improvement of bovine semen quality and resistance to oxidative stress through antioxidants. Detailed research was conducted on oxidative stress, resveratrol, melatonin, quercetin, nanoparticles, glutathione, vitamin E, and their relation to bovine semen. A diversity of articles and publications from 2019 to 2025 was selected from different scientific journals. The search platforms used were *PubMed* and *Scopus*. The quality of the materials used was evaluated thoroughly, with the quality of the journals and websites classified between Q1 and Q2 according to the *Scimago Journal and Country Rank*. Improvement of semen parameters was shown to be possible with antioxidant supplementation through diet and semen extenders. Currently, the most researched antioxidants are resveratrol and melatonin. The application of nanotechnology in semen extender supplementation seems to be a very promising practice. The study of new combinations and strategies for semen extender supplementation will provide valuable insights, paving the way for advancements in cattle production.

Keywords: semen, oxidative stress, antioxidants, viability, motility

1. Introduction

Spermatozoa's redox-regulated reactions affect their functionality, being fundamental in capacitation and hyperactivation [1]. During cellular metabolism, this gamete produces reactive oxygen species (ROS) that affect its structure and function. This can be attributed to the interaction of ROS with lipids, DNA, proteins, and other macromolecules. Besides the endogenous production of ROS, spermatozoa

are also exposed to exogenous ROS from leukocytes and immature and/or abnormal spermatozoa. Although spermatozoa require ROS for their functionality, they are very vulnerable to ROS because they have a plasma membrane rich in polyunsaturated fatty acids (PUFAs) [2].

To balance the exposure of the spermatozoa to ROS, the seminal plasma is rich in antioxidants, enzymes, and molecules that neutralize the action of ROS [1]. When the balance between ROS and antioxidants is interrupted and the quantity of ROS is elevated, oxidative stress occurs. Oxidative stress has a negative impact on semen quality [3].

ROS quantity can increase and lead to oxidative stress in semen samples as a consequence of extrinsic factors affecting the bovine organism. Examples of this are climate change and seasonality, pollution, human disturbance, and sample manipulation during assisted reproduction techniques (ART) [4].

Climate change and seasonality are causes of heat stress. With the increase in environmental temperature, testicular temperature also increases, leading to oxidative stress in this tissue. Heat stress leads to low sperm quality by increasing ROS production and consequent lipid peroxidation and DNA damage. Due to the oxidative stress created by heat stress, bovine sperm is primarily affected by high peroxidation in the tail region, which impacts the contractility mechanisms [5].

Sperm handling and storage in ART raise the probability of oxidative stress. Thermal changes, centrifugation, and freezing–thawing procedures can damage sperm and elevate susceptibility to oxidative stress – a risk that is aggravated if the seminal plasma, the natural antioxidant defense of sperm, is swapped for medium during ART [4]. The solution to counteract the possibility of oxidative stress in ART lies in supplementing the medium used for spermatozoa with antioxidants. Many types of antioxidants have been tested to improve semen quality, an example of which is through extenders in semen cryopreservation [6, 7].

2. Methodology

To better understand the current advances in the improvement of bovine semen resistance to oxidative stress, thorough research was conducted. A variety of articles and publications from 2019 to 2025 were selected from different scientific journals. After primary research, the antioxidants chosen to be referred to in this chapter were: resveratrol, melatonin, quercetin, nanoparticles, glutathione, and vitamin E. The platforms *PubMed* and *Scopus* were used for the research of materials related to oxidative stress in cattle semen, solutions for improving cattle semen quality, and the aforementioned antioxidants and their use in the enhancement of bovine semen. To ensure the quality of the information used, the inclusion criteria were the classification of journals and websites between Q1 and Q2 according to the *Scimago Journal and Country Rank*. Articles and publications from sources not classified in the *Scimago Journal and Country Rank* or with classifications below Q2 were excluded from this study.

3. Resveratrol

Resveratrol is a polyphenolic compound composed of a stilbene base connected to two phenolic rings linked by a styrene double bond, with the International Union of

Pure and Applied Chemistry (IUPAC) nomenclature: 3,4,5-trihydroxystilbene. It occurs in trans and cis isoforms, although trans-resveratrol is the most predominant in dietary sources and supplements [8]. Resveratrol can be found in multiple types of plants, and it has many properties, one of them being its antioxidant capacity. The antioxidant effect of resveratrol lies in the redox property of phenolic hydroxyl groups, which can act as free radical scavengers and activate many antioxidant enzymes [9].

Supplementation of resveratrol in cattle through feeding has been demonstrated to affect reproduction. In males, resveratrol could increase testosterone production and consequently enhance spermatogenesis and stimulate penile erection [10]. In ART, due to the induction of oxidative stress through freeze-thaw processes, the capacity of resveratrol as an antioxidant supplement in semen extenders has been studied and proven to increase sperm quality post-thawing [9].

In a study by Assunção et al., the supplementation of resveratrol at a concentration of 0.05 mM achieved better results in sperm quality improvement than other concentration values [11]. Another study confirmed this finding by obtaining better results in motility and other cellular parameters after cryopreservation of semen when using concentrations of 0.1 and 0.01 mM of resveratrol supplementation. Resveratrol proved to be important in the phosphorylation of the AMPK protein, a metabolic motor regulator of sperm, allowing, through this mechanism, the improvement of sperm motility [12]. When comparing the effect of 0.05 mM resveratrol in conventional slow freezing and ultra-rapid freezing semen methods, conventional slow freezing showed improved sperm motility, membrane integrity, and fertility with the supplementation. Although the ultra-rapid freezing method with resveratrol showed low cryo-survival sperm and fertility results, it demonstrated a reduction in oxidative stress [13].

4. Melatonin

Melatonin is a hormone that plays an important role in regulating the circadian rhythm in animals. This hormone exhibits multiple capacities, including anti-inflammatory, anti-apoptotic, and anti-aging properties. Additionally, melatonin has the ability, in some tissues and cells, to act against ROS, exerting an antioxidant effect [14]. In the male reproductive system, melatonin has been proven to be an important antioxidant, capable of activating signaling pathways in Sertoli cells, aiding their protection against oxidative stress [15]. Melatonin also influences testicular growth and development and plays a critical role in hormone secretion and spermatogenesis [14].

The antioxidant effect of melatonin has also been studied in bull semen extenders. In a study on the effect of different concentrations of melatonin in bull semen diluent for cryopreservation, 0.3 mg/mL was the concentration with the most positive effects on semen post-thaw. Antioxidant and anti-apoptotic abilities of sperm increased, sperm motility, acrosome, and plasma membrane integrity were improved. Additionally, sperm fertility capacity improved with the 0.3 mg/mL concentration of melatonin [16].

In sex-sorted bull semen, incubation in fertilization medium with a 2.3×10^{-5} M (0.1 mM) concentration of melatonin was demonstrated to significantly

decrease ROS levels and promote the antioxidant abilities of sperm. Acrosomal integrity and fertilization ability also improved with this concentration of melatonin [17]. The same concentration of melatonin, 0.1 mM, was also proven to be effective as a supplement in cryopreserved bull semen samples, improving post-thaw quality of semen, protecting the plasma membrane, the acrosome region, and maintaining the integrity of sperm ultrastructure [18]. In another study, thawed bull sperm incubated in medium with 100 μ M (0.1 mM) of melatonin presented better kinematic parameters and higher motile and rapid spermatozoa percentages. The supplementation of melatonin also showed greater viability and antioxidant status of sperm [19]. In another study, melatonin sperm supplementation improved sperm quality. The concentration of 10^{-3} M (100 mM) reduced the ROS levels to almost 50% in bull semen during *in vitro* fertilization (IVF) [20].

5. Quercetin

Quercetin is a flavonoid with the IUPAC nomenclature: 3,3',4',5,7-pentahydroxy flavone and is an antioxidant present in fruits and vegetables [21]. Besides its strong antioxidant capacity, quercetin also has anticarcinogenic, anti-inflammatory, and antimicrobial properties. The antioxidant effect of quercetin is considered very potent, having a strong capacity to scavenge ROS. The implementation of this antioxidant in semen extenders for a variety of animals has proved beneficial. In the bovine sector, the most recent studies on quercetin use in semen extenders were conducted in buffalo bull species. The sperm of buffalo bulls is high in PUFAs, making it more susceptible to ROS [21–23].

In buffalo bulls, quercetin supplementation in semen extenders improved post-thaw semen quality. With the use of 150 and 200 μ M of quercetin prior to dilution and cryopreservation of semen, better results were obtained showing superior semen quality parameters as seen in the study by Ahmed H. et al. [21]. In another study, the incorporation of 10 μ M of quercetin after thawing improved buffalo semen characteristics. This antioxidant concentration for post-thawing semen could lower oxidative stress derived from freezing [22].

In a study with Nellore bull semen, the supplementation prior to cryopreservation of quercetin, in concentrations of 25, 50, and 100 μ g/mL, did not show significant improvements in thawed semen parameters. However, the results were justified by the high quality of semen used in this study, leading to less differentiation between the control and quercetin groups [23].

6. Nanoparticles

One of the major recent advances in science is nanotechnology, which can be applied in different fields such as agriculture, livestock, and medicine. Nanominerals have proved to be an alternative to inorganic and organic minerals for animals, enhancing bioavailability and reducing pollution. Nanominerals have the capacity to promote antioxidant activity, improve reproductive performance in animals, and have the potential to aid in the improvement of ART [24]. In a study by Mousavi et al., the reduction of lecithin

particle size in semen extenders to a nano-level increased the performance of cryopreserved Holstein bull semen, proving that by reducing the particle size of the commonly used lecithin-based extenders, the efficiency of lecithin during cryopreservation was improved [25].

The potential of silver nanoparticles in semen extenders has also been studied. The main conclusion is that this nanoparticle can act as an alternative antibiotic agent without a significant cytotoxic effect on cryopreserved sperm [26]. In another study, the antibiotic property of silver nanoparticles was confirmed. However, using a supplementation of 125 µg/mL silver nanoparticles in the cryopreservation of bull semen also demonstrated a reduction in lipid peroxidation levels and ROS. That way, silver nanoparticles exhibit not only antibiotic properties but also a strong antioxidant capacity [27].

Selenium nanoparticles' potential in bull semen cryopreservation extenders has also been studied. In Holstein Friesian bulls, the concentration of 1.0 µg/mL of selenium nanoparticles improved post-thaw sperm parameters. These nanoparticles were able to reduce apoptosis, lipid peroxidation, and sperm damage due to cryopreservation thereby concluding that selenium nanoparticles have a positive impact on antioxidant sperm activity [28]. In another study, selenium nanoparticles were combined with glutathione in bull semen extenders to enhance the antioxidant potential of glutathione, which promoted better sperm parameters [29].

7. Glutathione

Glutathione is an enzymatic antioxidant present in bull sperm. The reduced form of glutathione scavenges electrophilic species and oxidants via catalysis. By having the capacity to protect cells from free radicals, ROS, lipid hydroperoxides, xenobiotic toxins, and heavy metals, glutathione is believed to suppress damage and improve the post-thaw quality of bull semen [30].

Supplementation of 1.0 mM glutathione peroxidase in a nano-lecithin-based extender presented a great percentage of plasma membrane functionality and integrity in bull sperm, proving also that this supplementation of enzymatic antioxidants improves post-thaw quality and *in vitro* fertility of bull sperm [31]. In another study, reduced glutathione supplementation in an egg yolk-based extender for cryopreserved bull semen proved to have the potential to improve embryo development [32].

In more recent studies, the supplementation of glutathione in bull sperm has been studied through nanoparticles. The use of nanoliposomes measuring 50–100 nm, loaded with 2.5 mM of glutathione in lecithin-based freezing extenders, proved to enhance bull semen cryopreservation. This conjugation of glutathione with these nanoparticles showed higher viability and plasma membrane integrity of sperm [33]. As referred to in the nanoparticles segment of this article, the co-supplementation of glutathione and selenium nanoparticles has been shown to have a positive effect on bull semen. Supplementation of 4 mM of glutathione with 2 µg/mL of selenium nanoparticles was able to enhance sperm motility, viability, mitochondrial activity, plasma membrane, and acrosome integrity [29].

8. Vitamin E

Vitamin E is a non-enzymatic antioxidant that acts principally against ROS and lipid peroxidation in sperm. It is considered a second-line defense antioxidant, with some studies presenting lower antioxidant capacity in comparison to others, such as glutathione [31]. However, the antioxidant capacity of vitamin E exists and has been studied, for example, through dietary supplementation. In a study by Yuan et al., dietary supplementation of vitamin E and flaxseed oil in bulls was able to improve motion parameters and IVF rates in frozen-thawed samples, which enhanced the embryonic development rate, justified by the propionic acid metabolism pathway [34].

Vitamin E can be used as an enhancer of other antioxidants. In Achai bulls, the supplementation of glycine was proven to obtain better results when coupled with vitamin E. The total antioxidant capacity of cryopreserved semen and conception rate were improved with the addition of glycine and vitamin E [35].

Although it has a powerful antioxidant effect, vitamin E shows limitations in its application to sperm cells. It was found that applying nanotechnology to vitamin E could reduce oxidative stress in sperm. Hence, supplementation of sperm with nano-emulsions along with vitamin E was able to improve motility parameters and reduce ROS and lipid peroxidation [36].

9. Discussion

Artificial insemination is a common practice in cattle production that leads to the need to develop methods that can optimize sperm quality, especially when it is cryopreserved. Sperm is highly affected by oxidative stress, with high quantities of ROS and lipid peroxidation being the main causes of sperm damage after thawing [6].

Different studies have been conducted on the effect of various antioxidants in bovine semen. Improvement in semen parameters has been shown to be possible with antioxidant supplementation through diet and semen extenders.

Most recent studies on antioxidant supplementation in bovine semen have highlighted the potential benefits of two promising antioxidants: resveratrol and melatonin. Resveratrol supplementation has been shown to be possible through diet and semen extenders [9, 10]. Semen extenders supplemented with 0.05 mM of resveratrol have demonstrated better results in some studies [11, 13]. Melatonin demonstrates beneficial properties for the male reproductive system [14]. When used for supplementation in bovine semen extenders, the concentration of 0.1 mM is presented as the most advantageous in a variety of studies [17–19].

The antioxidant with less extensive research material was quercetin, with recent studies referring to the supplementation of bovine semen with quercetin through different methods. In this way, it is difficult to conclude and deduce the best concentration and method of quercetin supplementation in bovine semen. Profound research is needed regarding the supplementation of bovine semen with this antioxidant [21–23].

Application of nanotechnology in semen extender supplementation seems to be a very promising practice [24]. Nanominerals such as silver and selenium have shown antioxidant properties, reducing ROS and lipid peroxidation in bovine semen [27, 28]. Adapting the use of antioxidants with nanotechnology has also improved semen quality. By complementing nanominerals with other antioxidants or reducing the particle size of supplementing antioxidants, the antioxidant effect can be enhanced and semen parameters improved, as exemplified in glutathione and vitamin E studies [29, 33, 36].

Further research into the role of antioxidants in semen is essential to uncover their full potential. Understanding how these compounds influence sperm parameters will allow for more precise and effective supplementation strategies. By identifying the optimal supplementation strategies for enhancing semen quality through antioxidant intervention, researchers and practitioners can work toward improving reproductive outcomes. These strategies must focus on combining different antioxidants and the application of nanotechnology through nanominerals and nanoparticles of antioxidants. Continued study in this area will provide valuable insights into the best ways to increase sperm quality and fertilization, paving the way for advancements in cattle production.

10. Conclusions

Improvement of semen parameters has been shown to be possible with antioxidant supplementation through diet and semen extenders. Resveratrol and melatonin show great capacity for combating oxidative stress and improving semen quality. The application of nanotechnology in semen extender supplementation seems to be a very promising practice. The possibility of semen supplementation with nanominerals and the reduction of antioxidant molecular size leads to the potential for new combinations and strategies in semen extender supplementation.

Continued research into the role of antioxidants and the development of efficient supplementation strategies will provide valuable insights, paving the way for advancements in cattle production.

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Conflict of interest

The authors declare, for all purposes, the absence of any conflicts of interest related to this report.

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Section 2

Reproductive Biotechnologies in Animal Production Systems

Chapter 3

The Contribution of Reproductive Technologies to Dairy Cattle Genetic Improvement in the Amhara Region: Successes and Challenges in Research and Development Efforts

Assemu Tesfa, Birhan Kassa, Mengistie Taye, Ahemed Alkader, Demelash Aychilie, Zemenu Birhan, Tarekegn Demeke and Mesfin Lakew

Abstract

In the Amhara region, cattle genetic improvement has been conducted by the Amhara Agricultural Research Institute (ARARI) and the Amhara Livestock and Fishery Resources Development Office (LFRDO). The aim of the article was to evaluate the contribution of reproductive technologies to dairy cattle genetic improvement in the research and development sectors of the Amhara region. A total of 13 years of data from the LFRDO was used to analyze the successes of reproductive technologies in the livestock extension sector. Under the research system, the delivery rate (in percentage) through Estrus/Oestrus Synchronization and Mass Artificial Insemination (OSMAI) was reported to range from 4.93% at Andassa Livestock Research Center (ALRC) to 60.1% at Debre Birhan Agricultural Research Center (DBARC). The data from LFRDO indicate a calving rate (in percentage) of 44.46 and 44.84 through insemination done with conventional semen and bull service, respectively. In the region, sexed semen for females was used to increase the number of crossbred females, and the work done over one year revealed an overall conception rate (in percentage) of 42.09%, ranging from 19.1% to 79%, and the overall sex efficiency for female calves was 94.8%. The time of insemination after the onset of heat, body condition, breed, parity, and technical efficiency of the artificial insemination technicians were reported as factors for the success of OSMAI. The OSMAI, which targets increasing milk yield, would be better integrated with different actors in the dairy value chain, and accountable collaboration between the various actors participating in the technology should be realized.

Keywords: Amhara region, conception rate, reproductive biotechnology, sexed semen, sex efficiency

1. Introduction

The demand for animal-source foods is anticipated to increase due to the growing world human population, projected to reach 9.7 billion by 2050, coupled with rising household incomes and urbanization [1, 2, 3]. Beyond the population increase, global food production is facing challenges due to climate-related issues, which necessitate a doubling of food production to meet the increasing food demand [4, 5, 6, 7]. Worldwide, animal production is expected to grow by 70% in order to meet the demands for animal-source foods and ensure food and nutrition security in both developed and developing countries [8]. Rapid genetic improvement through animal breeding, made possible by reproductive biotechnologies and genomic tools, is a key part of ensuring future food security [2].

Millions of people depend directly on indigenous animal genetic resources, which are believed to preserve a large portion of the current global genetic diversity [8, 9] and they are the only options available to millions of farmers in African agro-pastoral systems, where exotic breeds underperform in traditional management systems [10]. Because these breeds are the source of vast environmental adaptations for various diseases and parasite resistance, tropical climate conditions (including dry, hot, and humid), and excessive heat tolerance, they are suitable for extensive management systems and are the only options for ecosystem restoration [8, 11, 12].

Although the contribution of the livestock sector to the national economy in Ethiopia is significantly high, reaching about 25% of the total agricultural GDP [13], the milk production per animal is 1.45 liters per cow per day [14]. The average lactation milk production of indigenous cows ranges from 494 to 850 kg under optimum management [15], and the lactation length and average milk yield per day per cow were seven months and 1.48 liters, respectively [16]. Different efforts have been made to improve the milk productivity of these indigenous cattle resources through selection and crossbreeding. The presence of a range of agro-climates and production systems, feed and water availability, and small-to-large-scale farms are the best contributors to improving the genetic merits (Shapero [17] and IBC [18]); still, the required output was not attained. Some of the reasons for this are separate implementation interests between research institutes and development officials, lack of a clear and implementable strategy, and absence of sustainable linkage among different stakeholders [17].

In support of Ethiopia's dairy cattle genetic improvement, various reproductive biotechnological tools have been demonstrated. Among these, artificial insemination (AI) using exotic semen began in the 1960s [19]; subsequently, estrus synchronization was implemented in 1980 [20] throughout the country to assist AI activities. Embryo transfer (ET) was demonstrated at the International Livestock Research Institute (ILRI) and Holetta Agricultural Research Center (HARC) in 1990 [21] (Daba et al., [22]). Recently, the use of sexed semen in female calves has brought tangible results. AI and estrus synchronization have been widely implemented in research and development institutions throughout the nation, including the Amhara region, with the exception of ET, which is limited to research centers. To increase the productivity of crossbred animals, mass synchronization with fixed-time AI was recently implemented throughout the country.

Despite long-lasting efforts implemented through these technologies, the contribution of crossbred animals to the total cattle population is only 2.29% [16]. Although the Ethiopian government plans to increase milk yield by 200% and the number of crossbred animals by 600% by 2030 [23], the efficiency of these efforts so far in attaining the plan is below expectations. Several scholars have attempted to evaluate the efficiency of these technologies with different setups and animals of different breeds (blood levels), ages, and body condition scores (BCSs). This study aimed to evaluate the attempts made to assess the reproductive technologies implemented in dairy cattle genetics and identify the major hindering factors to recommend improvement directions for future implementations in the Amhara region.

2. Materials and methods

2.1 Description of the study area

The work was targeted to evaluate the reproductive technologies implemented in the Amhara Region, Ethiopia. The region is situated between 8°45' to 13°50'N and 36°20' to 40°30'E, with a land area of 170,752 square kilometers (see **Figure 1**). The region's capital city, Bahir Dar, is located 566 km from Addis Ababa. The region has 17.3 million cattle, contributing about 24.6% of the nation's cattle population [16]. The Amhara region holds the largest milk shed in the country, characterized by the presence of a large market, high potential for roughage production, a significant number of milk cooperatives, and cooperative unions involved in milk processing [24]. Menale et al. [25] reported that the biophysical environment of the region is approximately 14.2%, 56.1%, 28.1%, and 1.7% highly suitable, moderately suitable, marginally suitable, and unsuitable for dairy cattle production, respectively. According to Moges et al. [26], in the main towns of the region, 885 dairy farms were reported holding 1–2 cows, 3–10 cows, and > 10 cows per farm, with respective percentages of 45.1%, 46.2%, and 8.7%. In regions such as the Amhara LFRDO, Bahir Dar Artificial Insemination Center, Bahir Dar Animal Health and Disease Surveillance Office, Amhara Agricultural Research Institute (ARARI), and Amhara Cooperative Agency, these institutions work together on dairy cattle genetic improvement through different levels of participation and contributions.

2.2 Data source and analysis

To compile the study results, research findings on assisted reproductive technologies from research centers in the Amhara region were gathered. Thirteen years of retrospective data collected from the region's livestock extension system were used to evaluate the trends in the success of reproductive technologies in the region. The major working procedures and challenges related to the OSMAI in the Amhara region were collected from key informant interviews conducted with animal breeding experts in the office. In addition, a field visit was conducted to evaluate the performance of the Bahir Dar Artificial Insemination Center on semen collection, processing, and dissemination, their performance in liquid nitrogen production, and their involvement in the cattle genetic improvement program. The collected raw data were analyzed using Microsoft Excel software (MS-Excel, 2010).

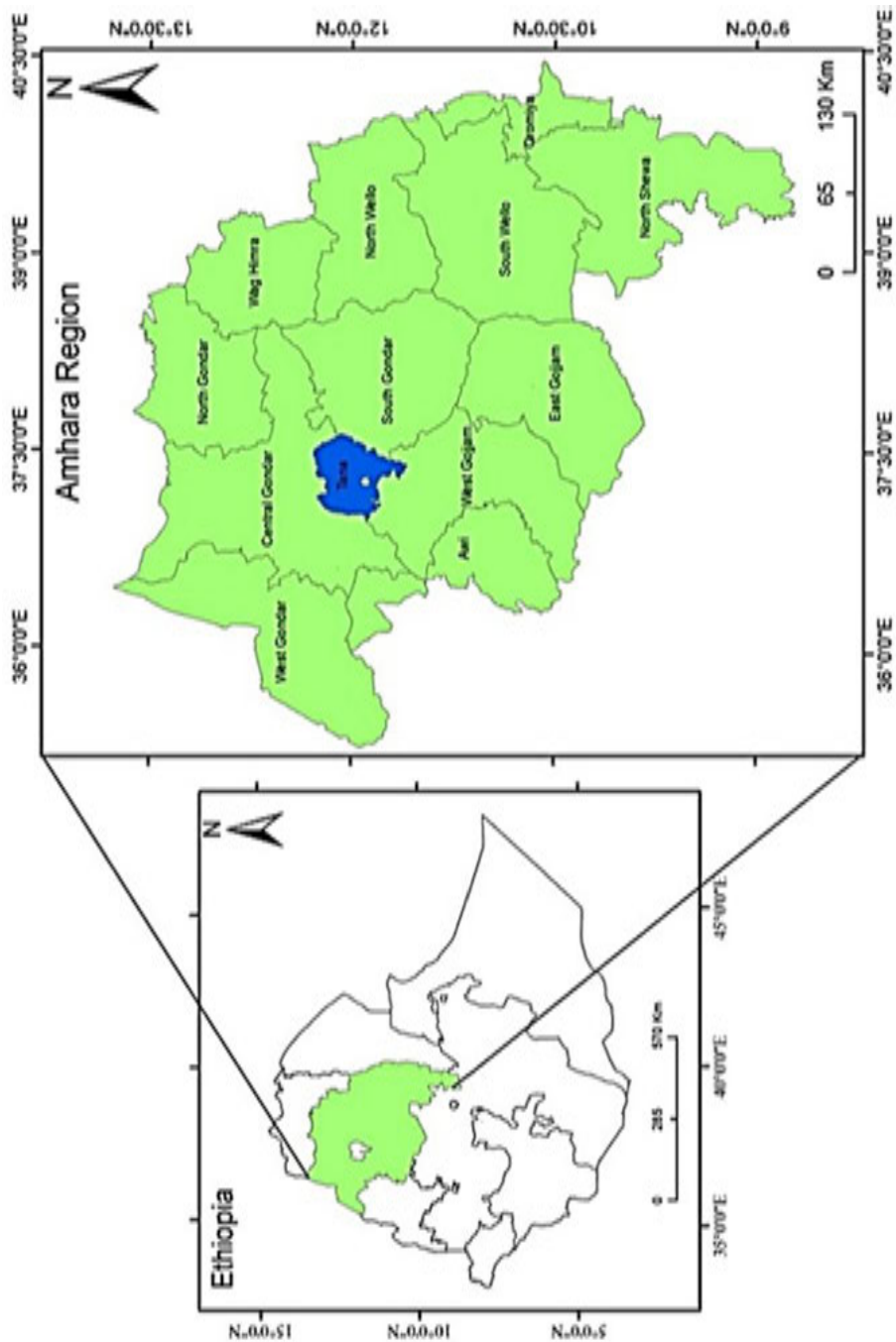


Figure 1.
Map of the Amhara region.

2.3 Estrus/Oestrus synchronization and AI protocols

Currently, prostaglandins and progesterone are widely used to synchronize estrus in dairy cattle operations (Murugavel et al., [27]). Prostaglandin is the first method of estrus synchronization that depends on the presence of a functional corpus luteum (CL), particularly in the diestrus stage of the estrous cycle (7th to 17th day of the cycle; Cordova-Izquierdo et al. [28]). The use of prostaglandin or its analog (PGF2 α) in a synchronization protocol shortens the estrous cycle, with estrus occurring two to seven days after hormone injection [29]. Two protocols based on the prostaglandin hormone were evaluated in the research centers: (1) single-shot, delivering AI after seven days of hormonal injection; and (2) double-shot, with repeated hormonal injection after 7 days and insemination within 18 days [30]. The prostaglandin-based synchronization technique to support AI has previously been evaluated in four regions of Ethiopia for four consecutive years: Oromia (283,231 cattle), Amhara (69,207 cattle), Tigray (129,220 cattle), and the SNNP (129,545 cattle) [20, 29]. Based on the Ethiopia National Dairy Development Strategy (2022–2031), it is projected that the number of crossbred cattle will be 1.03 million head and the number of exotic cows will be 0.26 million head by 2030, which is a 329% and 700% increase compared to the base year, 2020 [31]. To attain this ambition, the country implements OSMAI as a basic strategy, as purchasing animals is more costly for smallholder farmers [20, 29, 32].

2.4 Semen collection, processing, and dissemination in the Amhara region

The AI center of the Amhara region, which operates under the region's Livestock and Fishery Resources Development Office (LFRDO), has been mandated to collect, process, and distribute semen, train and certify AI technicians, and purchase and distribute AI equipment and consumables within the region. The region had five liquid nitrogen production plants in Bahir Dar, Gondar, Debre Markos, Dessie, and Debre Birhan, which maintained semen before distribution to neighboring districts. The production potential of the liquid nitrogen processing plants varies from 9 L/h in Debre Markos to 3 L/h in the others, and the low potential was due to the lack of timely maintenance and the aging of the plants (e.g., the plant installed at Bahir Dar dates back to the 1980s).

Semen collection in the region was performed using a dummy cow and an artificial vagina. The semen collected before distribution was subjected to different processing, quality tests, and packing procedures (see **Figure 2**), and the processing operations were performed in an enclosed area, which was restricted to technicians who operated the activity.

3. Result and discussions

3.1 Reproductive technologies for cattle genetic improvement in Ethiopia

The Ethiopian cattle genetic improvement through different reproductive biotechnologies is supported by various projects, such as the ILRI and African Dairy Genetic Gain (ADGG) [33]. Accordingly, the government has established five cattle genetic improvement and multiplication ranches throughout the country,

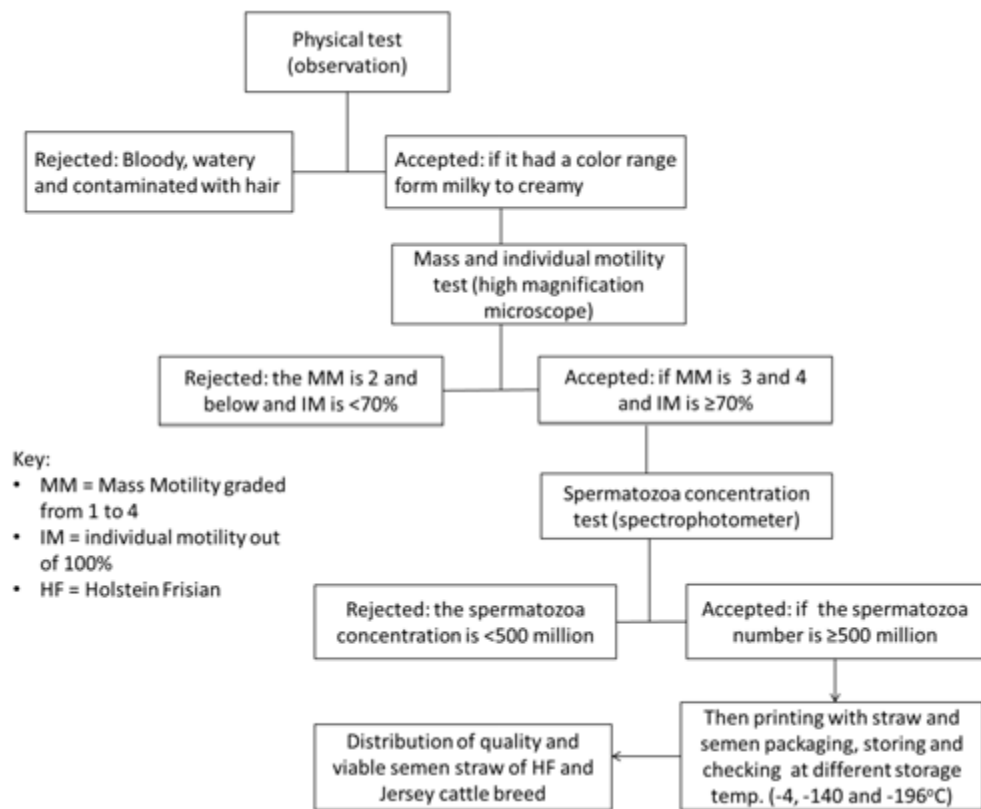


Figure 2.
Schematic diagram of semen collection, quality checking, and distribution used at Bahir Dar AI center.

which are used for the production of crossbred pregnant heifers and bulls to be distributed to smallholder farmers [34] and to act as test sites for different reproductive technologies. The first attempt at genetic improvement of cattle through crossbreeding was initiated by the Ethiopian Institute of Agricultural Research (EIAR) using Friesian, Jersey, and Simmental sires that were crossed with local Fogera, Horro, Boran, and Barka dams [19, 35, 18]. To date, three main reproductive technologies have been implemented in the country: AI, estrus synchronization, and ET [21, 36]. These have been implemented for cattle genetic improvement, with AI making the greatest contribution to genetic improvement programs [21].

ET, which is still limited to research centers, began in 1991 (Daba et al., 2022). In Ethiopia, the first calf from ET was born in 1991 at the ILCA-Debre Zeit Research Station (DZARC) from the production of eight pairs of identical twins using embryo-splitting technology (Daba et al., 2022). Another subsequent ET effort was restarted in 2009 at the Adami Tullu Research Center. Imported frozen embryos were transferred to Boran heifers as recipients, and six calves were born in 2010, after which the EIAR researchers produced three ET calves at DZARC, and two ET calves were born at HARC in 2010. At DZARC, an additional seven ET calves in the research center and two ET calves on dairy farms were born (Daba et al., 2022). Currently, the government has mobilized researchers to start ET technologies for

different selected local cattle breeds using the Livestock and Fisheries Sector Development (LFSD) project (LFSDP) and private organizations.

Hormone-assisted estrus synchronization in cattle was initiated in the late 1980s to introduce and integrate new reproductive technologies, such as ET, AI of local cattle with high-grade exotics, and to explore opportunities for using these technologies for genetic conservation of indigenous cattle resources [20]. Synchronization of estrus (heat) involves manipulating the estrous cycle of females to give birth at similar times, thereby saving both time and logistics (Rick and Gene, 2013 [37]). The technology also improves farm economics through shorter calving intervals, shorter breeding seasons, and reductions in time for estrous detection; it allows matching calving with feed availability and market demand for dairy products [20, 32].

3.2 Status of reproductive biotechnologies in research centers in Amhara region

In the Amhara region, a 77.0% estrus response under regular service and 89.3% under research conditions was achieved in cattle [36]. According to a report by Gatew et al. [38], the heat response in estrus synchronization was 74.48% in North Showa, 89.3% in the Bahir Dar milkshed [39], 100% in the Adigrat-Mekele milkshed [32], 97.7% in the Awassa-Dale milkshed, and 98.92% in the Fogera district [40]. These authors reported pregnancy rates of 20.18%, 13.17%, 61.7%, 57.7%, and 26.88%, respectively. However, lower and varying pregnancy rates (from 4% to 60%) have been recorded across research centers and extension works operating in the Amhara region. The heat response report indicated that the hormone induces heat in the animals, but the lowered pregnancy rate can be attributed to different factors, such as breed differences, body condition, artificial inseminator technician skill, and animal management before and after insemination.

In the Amhara region, beyond the work done by the LFRDO, the ARARI has conducted OSMIAI through its research centers for the last three years [41]. The institute cascaded the results obtained from the Debre Birhan Agricultural Research Center (DBARC), where a pregnancy rate of 60.1% was attained at three months of pregnancy diagnosis (PD) test [30]. Under the work done in the researchers' follow-up, various results, from a 4.93% delivery rate at Andassa Livestock Research Center (ALRC) [41] to 60.1% at DBARC [30], were reported (see **Table 1**). In a comparative study conducted by Shiferaw [42], in three districts – Bahir Dar, Mecha, and Yilmana Densa – with a total of 225 animals, a 66.15% estrus rate and a 36.27% pregnancy rate were reported in 2013, and the repeated study conducted in the same districts with 126 animals in 2014 showed an estrus rate of 88.9% and a pregnancy rate of 60.32%.

As presented in **Table 1**, the varying results between and among research centers, besides many other factors, might be due to the number of animals used. The higher number of animals reported, for example, in ALRC, makes addressing all animals for PD tests difficult, and the unwillingness of cattle-owner farmers to assess pregnancy contributed to lower conception rate reports.

3.3 Factors contributing to the success of OSMIAI under the research conditions

Researchers conducting OSMIAI in the Amhara region have reported various factors contributing to success (see **Table 2**). Yeshimebet et al. [30] reported that

Centers	Year	No. injected	No. in heat, N (%) ^a	No. inseminated, N (%)	No. conceived, N (%)	Birth, N (%)	References
DBARC	2018/2019	63	43 (68.3)	34 (79.1)	16 (47.1)	15 (93.8)	[29]
DBARC	2019/2020	395	243 (61.5)	181 (74.5)	66 (36.5)	64 (97)	[29]
DBARC	2015	94	89 (94.7)	89 (100)	53 (59.55)	50 (94.34)	[30]
ALRC	2019	395	241 (61.0)	224 (93.0)	NA	11 (4.93) ^b	[6]

^aPercentages (%) were calculated by dividing the active cell number by the previous column number (e.g., % heat = $43/63 \times 100 = 68.3\%$).

^bThe birth (in percentage) was calculated from the inseminated animals because pregnancy diagnosis was not done due to lack of permission from the farmers, and this contributes to the extremely lower delivery rate.

DBARC = Debre Birhan Agricultural Research Center; ALRC = Andassa Livestock Research Center.

Table 1.

Efficiency of estrus/oestrus synchronization and mass artificial insemination (OSMAI) activities in the research system of Amhara region.

Processes	Reasons for failures
Hormone administration (lower number of animals for OSMAI)	Animals that are pregnant, with a closed cervix, having low body condition scores, lower body weight, and those that are too old were not provided with the hormone
AI delivery (lower rate of hormonal response)	Animals failed to come for AI because of bull mating in the village, poor or inability to detect heat by farmers, the distance of the farmer's house from the AI center, and the farmers' need for the hormone (as they believe it brings non-cyclic animals to heat) were the major ones
Delivery rate (lower rate of conception and pregnancy)	The major probable reasons for the lower delivery rate were management effects (feeding and herding) after AI delivery, breed differences, inability to address all animals for pregnancy tests, and the absence of an animal's history after insemination

Table 2.

Major reasons contributing to the rates of insemination, conception, and delivery.

success was affected by the heat response duration (hours) after hormonal injection. The author indicated that cows inseminated within 72 to 120 hours of hormonal injection showed better heat response and pregnancy rates compared with animals inseminated at 24, 48, and 144 hours; and BCS, with animals having a score of 3.5 showing a better pregnancy rate than those with lower or higher BCSs. Belayneh et al. (2020) [40] reported that the low success rate of OSMAI was due to poor management of inseminated cows, inability to present efficient cows, inability to identify heat signs, poor capacity and absence of AI technicians at the time of heat, and the distance of hormone-injected cows from the collection centers, in their order of importance. Different factors were considered in the evaluation of OSMAI (see **Figure 3**) [42]. According to the author, age, body weight, and year also had an effect on the success of OSMAI, with cows aged four to seven years and cows with an average weight of 300 to 330 kg being the best achievers. Shiferaw [42] additionally

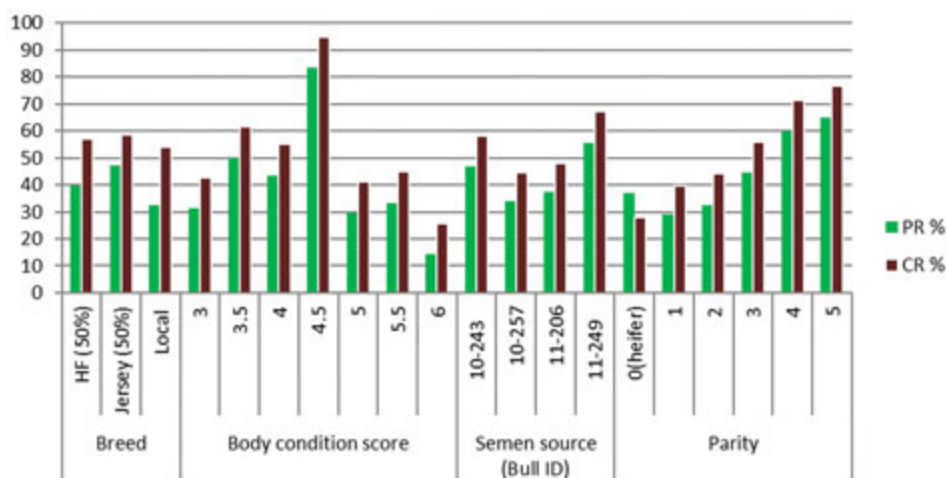


Figure 3.
Factors contributing to the success of pregnancy rate (PR%) and conception rate (CR%) through OSMIA under researchers' management: $CR\% = (\text{number of cows pregnant} / \text{number of cows inseminated}) \times 100$; $PR\% = (\text{heat detection rate} / \text{conception rate}) \times 100$; $\text{heat detection rate} = (\text{number of cows inseminated over 21 days} / \text{number of cows eligible to be bred over 21 days}) \times 100$.

reported that the variation in conception success due to technicians was significant and ranged from 16.03% to 40% when evaluating six technicians working in the Amhara region. Demis et al. [29] indicated that heifers had better conception rates than cows, and animals with a BCS of three (out of five) had comparably better conception rates. In addition, Marques et al. [43] indicated that the pregnancy rate increased with an increase in BCS from two to four. The major reasons contributing to the rates of insemination, conception, and delivery are summarized in **Table 2**.

3.4 Status of reproductive technology under LFRDO of the Amhara region assisted reproductive dairy technologies in the region

3.4.1 Status and achievement of AI

In the Amhara region, based on focus group discussions with regional experts, the mating of animals was conducted using AI and bull services with high-grade Holstein Friesian (HF) bulls. Bull mating was carried out in milkshed areas where AI services are not readily accessible and in peri-urban dairy cattle production belts. According to the regional LFRDO report, the number of cows served by AI and bull services, along with their respective calving rates across years, is presented in **Figure 4**. To increase the number of animals coming into heat, the region has continuously distributed hormones for estrus synchronization since 2012, and mating has been performed using either AI or bull mating.

The number of cows inseminated by AI and bull services has increased over the years, which indicates the growing need for crossbreeding animals and better achievements in the regional livestock sector to enhance milk production. However, the calving rate (%) with AI and bull service was inconsistent, with overall percentages of 44.46% and 44.84%, respectively (see **Figure 4**). The calving rate (in percentage) trend (see **Figure 4**) shows an inconsistency up to 2016, after which it

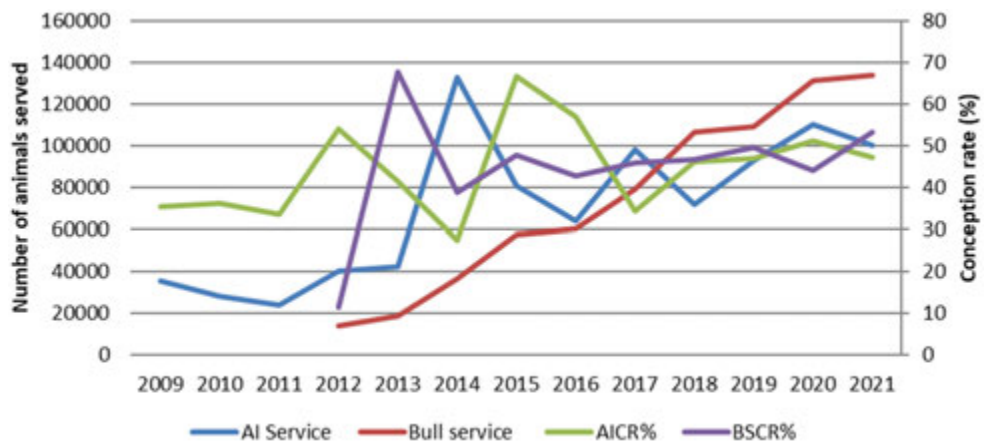


Figure 4.

Number of animals served through AI, bull and conception rate through AI (AICR%) and bull (BSCR%); AICR% = number of cows pregnant/number of cows inseminated with AI * 100; BSCR% = number of cows pregnant/number of cows served with bull × 100.

becomes a consistent and increasing trend, possibly due to the involvement and engagement of livestock experts in the genetic improvement program. Discussions among livestock office experts highlight that the lower success rate of AI is associated with the poor efficiency of artificial insemination technicians (AITs), poor semen handling and administration, inadequate cow management before and after insemination, and silent heat in zebu cattle. Similarly, Gebremedhin [44] stated that the success of AI depends on various factors such as the efficiency, capacity, and commitment of AI centers in procedurally and ethically producing, processing, handling, and distributing semen; the commitments and efficiencies of AITs; the presence of an appropriate breeding policy along with proper control of indiscriminate crossbreeding; and proper heat detection by farmers.

Based on the data, the number of animals served with mating crossbred bulls increased from 2012 to the present, while there was an inconsistent trend in OSMAI throughout the year (see **Figure 4**). It was observed that the average conception rate through bull services was higher than that through AI services. Although AI has a 41% advantage over natural mating through heat detection and timed AI [45], the conception rate in bull service was higher, which might be due to the correct heat detection by bulls and a heavier dose of spermatozoa per ejaculation.

3.4.2 Status of OSMAI

Estrus/Oestrus synchronization (OS) in the Amhara region was started in 2012 as a pilot study in four districts with the full involvement of researchers in a project-based study. In 2013, pre-scaling up was conducted in six districts, and in 2014, scaling out was conducted in 51 milkshed areas of the region. At the time, 67,852 animals were addressed with OS. However, as the work was conducted in a campaign without attention to basic contributing factors for the success of the OSMAI, the result was lower than expected. After gaining experience over these years, LFRDO lowered the number of districts and focused on milksheds that are well-established and suitable for monitoring to implement the technology.

The overall hormonal delivery of OS in the region showed a declining trend (see **Figure 5**), which can be attributed to different reasons. From this, the recurrent war starting from 2018/2019 resulted in low participation in projects that supplied hormones and other materials, a low level of participation by agricultural extension experts, and a lack of budget, as the government's recurrent budget had shifted to military support. All directions were toward safeguarding the country rather than working on genetic improvement. In addition, the work has started to be led by experts rather than politicians, with the involvement of research centers and universities and supporting projects (such as LFSDP, SNV, PAID), probably improving the achievement of OSMAI to increase the number of heifers and productivity per animal.

The one-year plan, 2022/2023, for the OS hormone service across zones of the Amhara region indicated an improvement in achievements to attain the plan (see **Figure 6**). From the graph, it was observed that West Gojjam and South Gondar are the best achievers of their plan in the half-year of 2022/2023. In addition, this might be due to these zones being well known for their suitability for dairy cattle (Minale et al., 2023 [25]) and having well-developed milk cooperatives with a large number of members.

3.4.3 Status and achievement of sexed semen

The first live calf through sexed-semen was born in 1993, following the advent of flow cytometric sorting in bovine spermatozoa in the 1980s (Reese et al., 2021 [46]). Sexed semen in the Amhara region was introduced at the research level in 2014 by researchers from ILRI and the ALRC. However, the success rate was low because the evaluation was conducted on local Fogera cattle, and there was improper handling of inseminated cows, which were subsequently mated with local bulls. Subsequently, AI with sexed semen was initiated in 2020 under government ranches to produce and distribute in-calf crossbreed heifers to local producers. This strategy was also implemented throughout the country as part of the *YeLemat Turufat* project, launched by the Prime Minister on November 3, 2022, for four consecutive years

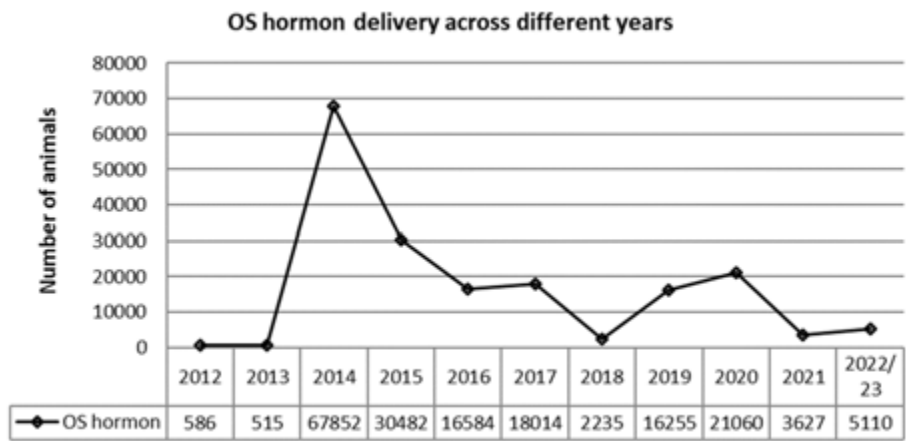


Figure 5.
Achievements of OS hormone delivery in the Amhara region across different years.

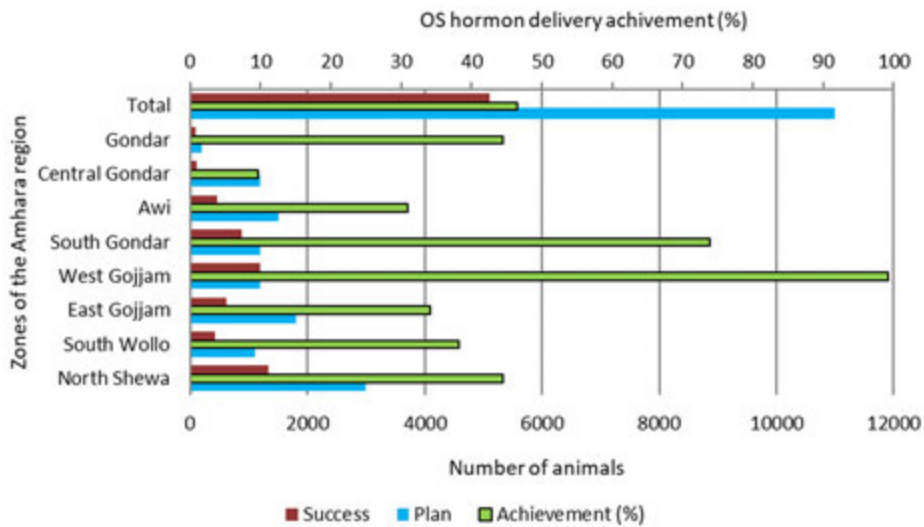


Figure 6.

The number of cows/heifers planned to be administered OS hormone and their success across zones of the Amhara region in 2022/2023 under LFSDO; achievement (%) = success/plan × 100.

[47]. The main application of sexed semen is to increase the percentage of heifer calves to expand herds or to sell replacements. In the Amhara region, it was offered at a subsidized cost (1,000 Ethiopian birr (ETB) per straw) through the LFSDP and PAID projects in a selected and well-established milkshed area.

In the work done during the 2022 fiscal year, the overall conception rate (in percentage) of AI with sexed semen was 42.09%, ranging from 19.1% at Chagni Ranch and 22.2% in Dangela Town to 64% in Kombolcha Town and 79% at Bahir Dar Town intensive dairy farms. The overall sex efficiency for females of the semen was 94.8%, with lower efficiency in Gondar (71.4%), Debre Tabor (75%), and Kombolcha (75%), while 100% female calves were delivered at Chagni Ranch, South Achefer District, Debre Markos Town, Mekit District, Bahir Dar Town, and Dessie Town (see **Table 3**). Compared to the potential sex efficiency of sexed semen (90%; Kumar et al., 2017 [48]; Reese et al., 2021; Boneya, 2021) the current findings are comparable to and above the potential average of the technology. The reason for low sex efficiency in the aforementioned districts might be the repeated breeding nature of the cows, in that the producers use conventional unsexed semen for the second round of insemination due to the cost and limited access to sexed semen. The conception rate observed in this study (35.5%) was lower than the reported average for the technology, 50%–60% [43]. This might be due to sexed semen being affected during the sorting process, the low number of spermatozoa per straw (5 million vs. 20 million in conventional semen), confirmation of active heat, inseminator skill, timing of AI, and other factors. Although the producers need and are eager to use the semen, its cost is 5,000 ETB per straw and is subsidized through projects to 1,000 ETB per straw, but its lowered conception rate and repeated mating requirements limit its wider application.

A meta-analysis conducted (Reese et al., 2021) on sexed semen indicated that the use of sexed semen reduces pregnancy rates by 23% (25% in cows, 21% in heifers) and calving rates by 24%. Boneya (2021) also reported that the biggest disadvantage

Location	No of cows inseminated	No of cows conceived	Conception rate (%)	Birth			Sex efficiency (%)
				Female	Male	Total	
Chagni Ranch	241	46	19.1	30	0	30	100
Dangila	99	22	22.2	16	1	17	94.1
S/Achefer	96	25	26.1	22	0	22	100
N/Achefer	90	36	40	36	1	37	97.2
Farta	217	51	23.5	12	2	14	85.7
Debre Tabor	29	11	37.9	3	1	4	75
Gonder	50	19	38	5	2	7	71.4
Anigolela	25	8	32	8	1	9	88.9
D/Birhan	25	12	48	7	1	8	87.5
Kombolcha	50	32	64	9	3	12	75
Debre Markos	29	17	58.6				
Mekit	51	27	52.9	5	0	5	100
Bahir Dar	100	79	79	57	0	57	100
Dessie	50	24	48	11	0	11	100
Average	1,152	409	35.5	221	12	233	94.8

Note: Conception rate (%) = number of cows conceived/number of cows inseminated × 100; sex efficiency (%) = number of female calves born/total number of calves born × 100.

Table 3.
Performance of female-sexed semen, conception rate (%), and sex efficiency (%) in major milkshed towns of the Amhara region in one year (2022).

of sex-sorted semen is its decreased conception rate. Similarly, Babura et al. [49] (2021) indicated that the use of sexed semen for embryo development contributed to a higher number of defective embryos compared to conventional unsexed semen, based on comparable work done on Boran and HF cross breeds in Bishoftu, Ethiopia. Babura et al. (2021) pointed out that the major issue with the low conception rate related to sexed semen was associated with proper handling techniques and the exact site of semen deposition.

3.5 Challenges in OSMAI under livestock extension (LFRDO)

The success of the OSMAI relies on four main pillars (see **Figure 7**), and they have contributed equally to its success. If there is a deviation in any of the components, the success will vary. According to discussions with the livestock office's genetic improvement experts, these factors, at different levels of effect, have contributed to the success of OSMAI in the region. According to a report from the office, more than 400 certified AITs participate in dairy genetic improvement programs through crossbreeding in the region. Most of them were trained and permanently recruited by the office; some of them are private, while others operate under milk cooperatives.

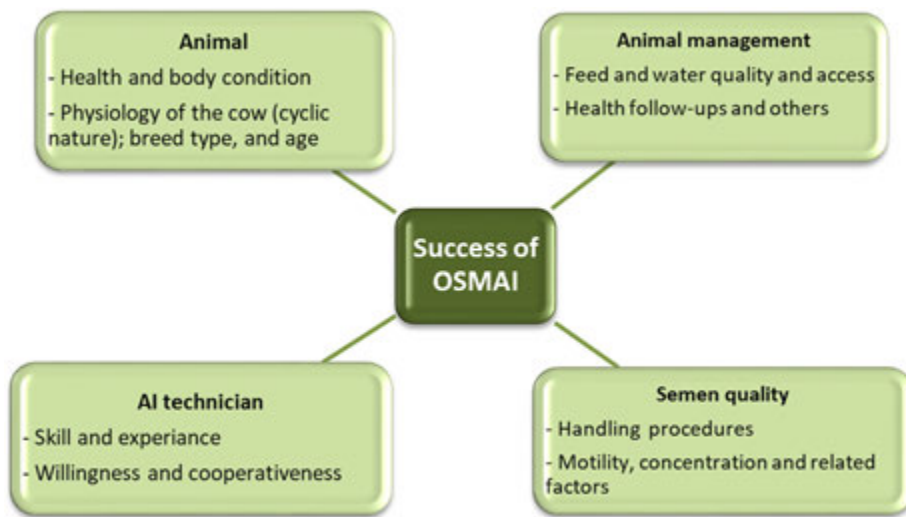


Figure 7.
The main pillars contributing to the success of OSMAl.

With the presence of these potentials in the region, the effectiveness and wide range of applications of OSMAl have been challenged by various factors. Limited structural and functional linkage and communication systems among AI centers and district-level LFRDO are the major challenges in establishing proper and structured data collection and reporting systems. The absence of incentives, rewards, and refresher training for AI technicians, coupled with the lengthy working steps of OSMAl (animal selection, identification of active CL, delivering hormones, following the heat, insemination, PD test), lowers the willingness of the technicians and also contributes to the disruption of AI delivery during weekends and holidays throughout the region. In addition, the following challenges are discussed:

- Lack and presence of variation among AI technicians in identifying the active CL before insemination to check the cyclic nature of the animals and detect active heat during insemination.
- Absence of collaboration between required actors, such as research centers, universities, and projects.
- Lack of integration with feed, animal health, better environmental conditions/season, and other required inputs.
- Pre- and post-cow management after OS hormone delivery and insemination: flushing cows with the best feed and separating the cow from the bull after hormonal injection and AI for two to three days is mandatory to avoid losing a crossbred calf.
- The hormone induces silent heat in indigenous cattle breeds, which requires diligent follow-up after hormonal delivery. Some technical and organizational problems associated with the success of OSMAl have been reported [20].

4. Conclusions and recommendations

The overall success rate of OSMAI in the region through research and development practitioners' management is good, but to further improve success, utilizing the available human resources, installed capacities, funding projects, and government direction is mandatory. The performance of liquid nitrogen plants in this region is very low, which poses a major threat to future genetic improvement programs. The most important challenges of OSMAI in the region are the low motivation of technicians, lack of inputs, inadequate liquid nitrogen, low engagement of stakeholders, lack of appropriate collaborations, and the absence of a herd recording system. The following recommendations were proposed to improve the current level of OSMAI success in this region:

- New semen and liquid nitrogen production and distribution centers should be established to satisfy the demand of livestock keepers.
- Wider awareness creation, proper training, careful animal selection, good animal handling facilities, and a well-trained, willing, and cooperative technician are mandatory.
- The OSMAI, which aims to increase milk yield, should be integrated with different actors in the dairy value chain, such as feed and medication suppliers, and around producers with strong dairy cooperatives.
- Strong and accountable collaboration between different actors participating in the OSMAI should be realized.

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Authors' contributions

Assemu Tesfa, Birhan Kassa, and Mengistie Taye were responsible for the study design, data collection, analysis, interpretation, and chapter writing. Ahemed Alkader, Demelash Aychilie, Zemenu Birhan, Tarekegn Demeke, and Mesfin Lakew participated in the raw data collection and preliminary data analysis. All the authors have read and agreed to the published version of the chapter.

Conflicts of interest

No potential conflict of interest was reported by the author(s).

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Section 3

Small Ruminant Production and Rural Livelihoods

Chapter 4

Husbandry Practices, Performance Potentials, Breeding Strategies and Socioeconomic Roles of Black Bengal Goat to Enhance the Food Security, Empower Women and Improve the Rural Livelihoods in Bangladesh

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Abstract

Black Bengal goat belongs to the Bovidae family and is found throughout the Bangladesh, West Bengal, Bihar, and Orissa regions of northeastern India. It is estimated that more than 90% of goat population in Bangladesh comprised the Black Bengal, the remainder being Jamunapari and their crosses. Higher prolificacy, fertility, resistance against common diseases, adaptability to the adverse environmental condition, early maturity, and superiority in the litter size are some of the outstanding features of Black Bengal goat. We have highlighted the Black Bengal goat genome, their production and management system including housing, feeding, nutrition, disease prevalence and control system, productive and reproductive performance potentials, present breeding scenarios and breeding strategies for genetic improvements, initiatives for the conservation, adaptation of modern reproductive biotechnologies, socioeconomic contribution to scale up the rural livelihoods, alleviate poverty, enhance food security and empower women in Bangladesh.

Keywords: goat production, husbandry practices, disease control, breeding strategy, socioeconomic condition, BBG genome

1. Introduction

Goats worldwide play a crucial role in smallholder farming systems, efficiently converting low-quality forage into high-value products and enhancing resilience

against economic and environmental challenges. They are socioeconomically important due to their adaptability, low feed needs, and short reproductive cycles, which help to grow goat populations and the industry worldwide [1]. Goat meat is widely accepted in many countries and religions [2], and the growing demand for goat milk, which is valued for its nutritional and therapeutic qualities [3], further highlights their economic significance.

Goat is now considered as the most promising livestock species for commercial meat (chevon) production [4]. The Black Bengal goat, which makes up more than 90% of the goat population in Bangladesh and is the only recognized indigenous breed, is raised in traditional village-based systems in more than 98% of cases [5, 6]. It is widely distributed throughout Bangladesh and northeastern India, including West Bengal, Bihar, Tripura, and Orissa [7]. Black Bengal goat (BBG) is famous for its high fertility, prolificacy, superior skin and meat quality, early sexual maturity, resistance against common diseases and short kidding interval [5, 8, 9]. BBG is a medium-type meat breed (birth weight ranges from 0.8 to 1.80 kg) [10–13], raised mostly in semi-intensive systems, fed with domestic food waste, crop waste, and natural plants. Goat farming is an accessible and financially feasible livelihood alternative for smallholder farmers because of traditional husbandry practices that include free-range grazing, low-cost feeding, and little veterinary care. Black Bengal goat's versatility makes it a perfect choice for agricultural systems with little resources. Tropical regions and marginal grazing areas are ideal for their growth [8, 14]. Its adaptability to harsh conditions is further supported by its capacity to use forages and resist disease stresses. The Black Bengal goat's ability to produce both meat and leather makes it an essential part of rural economies. Its early maturity, prolificacy, and low care needs guarantee small-scale growers a steady stream of revenue. The Black Bengal goat is essential to Bangladesh's socioeconomic environment because it supports rural livelihoods, food security, and women's emancipation.

Goat farming is particularly significant for women in rural areas, as it requires minimal initial investment, can be managed alongside household responsibilities, and is a reliable income source. Women's participation in goat rearing enhances financial independence, improves household nutrition, and provides economic stability, making it a key driver of poverty alleviation.

2. Evolutionary and historical background of Black Bengal goat

Among the first domestic animals, goats were kept by man in the Asiatic region of the world for its diverse products such as meat, milk, skin and fiber. The goat was the earliest ruminant to be domesticated [15]. Archaeological data suggested that goat was the first domesticated in an area around the Indus Valley in South East Asia [16]. The domestication and breeding of goat began in this region between 7500 and 7000 BC [17].

The Black Bengal Goat (BBG), a native breed of Bangladesh (**Figure 1**), has evolved through natural selection and adaptation to local environmental conditions over centuries. The wild bezoar goat (*Capra aegagrus*) is the ancestor of domestic goats, which have spread over various regions. The BBG, like other domesticated goats, may have descended from the *Capra hircus* species, which came from the Bezoar group's wild goats [8]. It is believed that BBGs have been introduced to the Indian subcontinent from Western Asia via migration routes through Afghanistan, Turkistan, and the Khyber Pass around 7500–7000 B.C. In South Asia, they adapted



Figure 1.
Black Bengal goat (BBG) male and female (Photocredit: Professor Dr. Auvijit Saha Apu), (a) BBG male, and (b) BBG Female.

to diverse climatic conditions and evolved into a hardy, disease-resistant breed known for its high-quality meat and skin. However, a recent study [18] on the phylogenetic status and genetic diversity of BBGs and Jamunapari goats in Bangladesh used the cytochrome b (cytb) gene of mitochondrial DNA. The study found 100% homology between BBGs from Bangladesh and India, as well as with goats from China, Thailand, Japan, and South Africa. The BBG and Jamunapari goats were closely related to goats from China, Pakistan, Slovenia, Switzerland, and other countries, sharing 98.3–99% similarity. The sequence data suggests a common origin between BBGs from Bangladesh, India, and China, indicating genetic linkage and evolutionary relationships among them.

3. Black Bengal goat genome

The Black Bengal goat (*Capra hircus*) is a popular goat in Bangladesh because of its adaptability, high-quality meat, and prolificacy. Researchers sequenced the organism's 16,640-base pair mitochondrial genome, which contains 37 genes, and created a draft genome assembly of roughly 3.04 gigabases with 26,458 annotated gene models in 2019 [19, 20]. Furthermore, about 9.4 million high-quality SNPs with 1 million indels and about 76,000 exonic SNPs influence meat quality, offering crucial genomic resources for functional genomics and breeding [21].

4. Goat population in Bangladesh

One of the most populated countries is Bangladesh, which is home to almost 175 million people within 147,500 square kilometers. However, most goats are raised by rural households, particularly by women and children. **Figure 2** illustrates the goat population in the country over the past decades [22]. Approximately 65% of households are involved in goat farming, either as a primary or secondary source of income [10]. On average, each farm has four goats, and in some regions, up to 41% of farm income is derived from goats [23] and contribute significantly to the national economy through meat, milk, and skin production, accounting for about 28%, 23%, and 28% of national outputs, respectively [24].

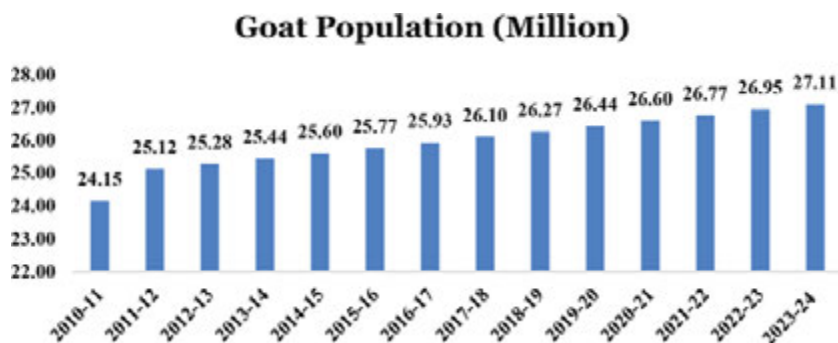


Figure 2.
Goat population in Bangladesh (source: compiled data of DLS [22]).

5. Husbandry practices and management system of Black Bengal goat in Bangladesh

5.1 Housing and farming systems

Three different farming systems are used by farmers: *Extensive system (ES)*: Free grazing on uncultivated land with little inputs, which results in a slow rate of weight gain. *Intensive system (IS)*: Long-term habitation with complete nutritional support; expensive but increases output. *Semi-intensive system (SIS)*: To balance cost-effectiveness and nutrition, SIS combines daytime grazing with overnight housing [25]. In the rural area of Bangladesh, most of the goat farming (67.3%) is semi-intensive, with 8.5% engaging in intensive farming and 24.1% utilizing free-range or extensive [26, 27]. By striking a balance between grazing and supplementation, the semi-intensive system maximizes productivity while keeping labor and input costs under control. Although intensive systems are becoming more popular as grazing pastures become smaller, the semi-intensive system is still the most popular and feasible.

Housing is crucial to goat husbandry. According to a study [28], 82% of farmers choose kacha (unimproved) floors, whilst 18% use paka (improved) floors. While roughly 50.8% build separate night shelters and 59.8% utilize corrugated steel sheets, straw bedding (47.5%) and earthen floors (61.8%) are more prevalent [26]. Just 12% of farmers utilize bamboo platforms, which compromise hygiene, whereas most farmers (75.6%) house goats at night for protection [27, 29]. To prevent cold stress, 93% avoid winter bathing, and 18% co-house goats with other livestock [27, 30].

5.2 Available feeds and feeding practices

BBGs in Bangladesh are mostly fed locally accessible green grasses, tree leaves, crop leftovers, and low-quality hay [31]. Grazing, tethering, semi-intensive, and stall-feeding are the four feeding techniques used to manage goats [32]. While 61% of farmers rely solely on green grass, 39% supplement homemade concentrates [33]. In a study [26], 34.2% of farmers just use pasture, 30.2% add concentrates to grass, and 53.8% permit grazing for 5–8 hours every day. According to another study [28] showed, 14% of farmers do not utilize concentrates with green grass, whereas 86% do. Additionally, some farmers add oil cakes, rice polish, and wheat bran for extra nutrients [34]. Growth and reproduction are improved by supplementation [25, 32].

Just 15% of farmers grow fodder, whereas the majority (76%) obtain green grasses from crop fields, roadsides, and vacant land [27, 35] and about 92% incorporate tree leaves into diets [36]. In commercial systems, 85% of farmers provide roadside grass, tree leaves, and natural grasses [35, 37]. Key feed sources included native grasses (*Digitaria sanguinalis*, *Panicum repens*, and *Cynodon dactylon*), tree leaves (Jackfruit, Mango, Shewra, Banyan, Jujube, Korai, Country almond, Banana, Coral tree, Indian Blackberry, Moringa, Guava, Bamboo), and crop by-products (Wheat bran, Khesari kalai, Mash kalai, and Rice bran) were identified by several investigators [38] in their evaluation of goat feeds and production potentials. BBGs are efficient consumers of these low-quality forages such as roadside grass, tree leaves, and crop residues [27].

According to a survey, just 4% of farmers grow fodder for goats, 96% utilize roadside grasses and tree leaves, 85% use premade mixed feed, and 11% supplement with vitamins and minerals [39]. This highlights the reliance on locally available, natural feed sources and the limited use of supplementation in traditional feeding practices. The nutritional status of BBGs in Bangladesh is often poor due to a shortage of quality feed resources, which are typically high in roughage but lack adequate protein, energy, and minerals [28, 40]. To increase body weight gain, condition score, and feed conversion efficiency, concentrate supplementation is crucial, particularly during the lactation and growth phases [41]. A concentrated mixture comprising 15.60% crude protein, given at 30% of total dry matter per 100 kg body weight for maximum growth, is recommended by some investigators [32] whereas other investigators [40] advised supplementing female goats with 250 g of concentrate daily. Nowadays, pellet feed adoption is increasing due to better feed conversion and cost-effectiveness [41]. Pellet feeds cut costs by 40%, and the growing use of ready-made feeds based on total mixed rations (TMR) improves dry matter intake (DMI), feed intake, and weight gain [41].

5.3 Effects of supplementation on growth and reproductive performance

Black Bengal goat (BBGs) in rural areas of Bangladesh suffer from poor growth and reproductive performance due to restricted supplementation, particularly when the animals are malnourished, which results in slower growth and decreased reproductive efficiency [28]. According to a study [41], pellet feeds considerably increase body weight gain, body condition, and feed conversion efficiency at a lower cost than conventional techniques, demonstrating the importance of concentrate supplementation for enhancing reproductive performance [32]. According to Devendra [42], tree leaves are also a vital source of nutrition. Growth benefits from supplementing with jackfruit leaves and mashkalai bran, with both improving daily weight increase and digestible nutrient intake [43]. Mango, Shaora, and ipil-ipil (*Leucaena leucocephala*) leaves have also been effectively combined with concentrate supplements [44, 45]. Supplementing with 100 g of Urea Molasses Blocks (UMB) per day enhances reproductive and productive performance [46]. Supplementing with pre-partum concentrate improves reproductive performance [47], and giving suckling goats 250 g of concentrate everyday increases productivity [40]. Farmers in the central region use concentrates at a rate of 82%, whereas farmers in the southern region use concentrates at a rate of 61% [29]. Better growth rates and carcass gain are the outcomes of a diet with greater protein content (20.3% CP) [48]. Growth and nutritional digestibility are enhanced by protein supplementation (16% CP) from soybean meal, rice polish, and wheat bran [49]. According to Uddin [50], concentrate combinations comprising soybean meal, rice polish, and wheat bran also promote growth in grazing

goats. Dietary level of energy (10.20–11.73 MJ/kg DM) positively influences ovarian morphological parameters and follicular development in Black Bengal goats [51]. On the other hand, dietary protein levels (18% CP) during late pregnancy influence milk yield, lactation length, and post-partum anestrus in Black Bengal and enhance the growth performance and survival rate of kids while influencing their hot carcass weight, dressing percentage, and fat content in their meat [52]. Soybean-based milk replacer can be fed as an alternative to goat milk without having any significantly negative effect on growth and blood parameters at the post-weaning stage of Black Bengal kids [53]. Moreover, feeding with soybean-based milk replacer enhanced the growth of kids comparing naturally reared counterparts. It is also recommended that soybean-based milk replacer feeding should be popularized to reduce kid mortality and improve the performance of BBG kids at the village level [54].

6. Productive performance potentials of Black Bengal goat

Birth weight is highest in single kids, followed by twins and triplets, and increases with the advancement of dam parity [55]. Kids birth weights are influenced by sex, season, litter size [12], dam parity [13]. The BBG has moderate daily gain (38.2 to 51.9 g/d), which is influenced by sex, birth type, birth weight, stage of growth, nutrition, season, rearing system, dam's weight, and body condition score of dam during kidding [47, 56–61]. According to studies, the breed's average birth weights are 1.03 kg [62], 0.96 kg [12], and 1.08 kg [63]. During the pre-weaning stage, kids raised in semi-intensive systems gain 33.08 g of weight per day under ideal circumstances, but kids raised in completely intensive systems gain 49.28 g [64]. Growth rate of BBG can be improved through a selective breeding program within the breed [65]. Body weight of BBG: three-month body weight ranges 4.05–4.27 kg [12], 4.24–5.84 kg [66], 5.02 kg [67], 4.8 kg [68], 6-month body weight ranges 6.99–7.11 kg [12], 7.28–9.19 kg [66], 8.41 kg [67], 8.0 kg [68], 9-month body weight ranges 10.29–12.89 kg [66], 10.41 kg [67], 11.1 kg [68], 12-month body weight ranges 13.52–15.37 [66], 12.42 kg [67], 14.0 kg [68]. At maturity, adult males typically weigh 17.9 kg, and females weigh 12.10 kg [68]. The optimum slaughter age for Black Bengal goats under semi-intensive management is around 9 months [69]. In case of Black Bengal goats, a positive correlation between birth weight and rate of growth, age at maturity and mature body weight with length, height and heart girth was found, which influences the future productive and reproductive performance of the animal [70]. There is a large variation within breed, and there are some individuals with outstanding performance even under the field conditions which might be the resources for breeders to develop this promising goat through planned breeding and selection [13].

While the Black Bengal goat is primarily a meat breed, it also produces little amount of milk. Various studies suggested that average milk yields for the Black Bengal goat: 303 ml [62], 158 ml [71], 275 ml [63], 214 ml [12], and 247 ml [72]. Milk yield is generally lower in the first parity compared to the third parity, as noted in studies by [73, 74]. Additionally, the body condition score of does play a significant role in milk production, with higher body condition correlating with better milk yields [62]. The milk produced by Black Bengal goats is rich in fat (5–7%), protein, and essential nutrients, making it highly beneficial for human nutrition, particularly for children and the elderly.

The superior skin of the Black Bengal goat is also known for its strength, elasticity, and beautiful texture. With its extensive use in the creation of high-end shoes, gloves,

and purses, this leather offers a valuable by-product that greatly boosts the income of small-scale producers and rural farmers. Black Bengal goat skin has an average weight of 998.80 g, height of 29.24 cm, and breadth of 20.91 cm [75]. Furthermore, the quality and characteristics of BBG skin gelatin were emphasized by some researchers [76], who proposed it as a viable substitute for commercially available beef and hog skin gelatins.

7. Reproductive performance potentials of Black Bengal goat

Reproductive efficiency is always considered to be the most vital factor ensuring increase in productivity to a certain environmental condition [77]. One of the most favorable attributes of the BBG as a meat-producing animal is its high rate of reproduction [78]. BBG is the most adaptable, widely distributed, and prominent goat breed in Bangladesh, well known in the world for its high prolificacy [79]. Among the small ruminants, BBG is the most prolific and able to breed throughout the year under tropical and subtropical regions of the world [80].

The BBG is also known for its early sexual maturity; males reach puberty at 6–8 months, while females reach it at 5–7 months. Their great reproductive efficiency is mostly due to their early maturity, which enables farmers to quickly complete several kidding cycles. When properly managed, the breed's pregnancy rates frequently surpass 90%, demonstrating its excellent fertility. Additionally, females can increase their contribution to the production of meat and skin by breeding twice a year [6, 8, 10, 14]. Average goat gestation period is fairly constant at around 146 days [8]; factors such as season, year, and sire can influence its duration [81]. Likewise, there has been no discernible impact of sex, birth type, or season on gestation time [12]. Some common reproductive parameters of BBG are listed in **Table 1**.

According to a study [82], kid mortality rates in semi-intensive conditions were $15.0 \pm 0.50\%$, while under extensive ones, they were $10.07 \pm 0.32\%$. Strong maternal instincts in BBGs lead to better parental care and increased survival rates. Kid

Parameter	Values (Mean $\pm$ SE)
Age at puberty (days)	197.82 $\pm$ 12.58 to 208.82 $\pm$ 12.60 [82], 199.32 $\pm$ 2.41 [66], 215.5 $\pm$ 15.6 [83]
Age at first conception (days)	292.96 $\pm$ 0.50 to 287.65 $\pm$ 0.52 [82], 217.94 $\pm$ 16.87 [71], 219.00 $\pm$ 8.66 to 225.45 $\pm$ 8.202 [84]
Service per conception (No.)	1.17 $\pm$ 0.04 [66], 1.25 $\pm$ 0.05 [83], 1.45 $\pm$ 0.1 [11]
Conception rate (%)	43.9% to 59.8% [85], 43.75% to 55.90% [86], 40.0% [12], 40.0–42.86% [87]
Age at first kidding (days)	448.26 $\pm$ 25.48 to 450.07 $\pm$ 22.43 [82], 368.12 [71] 371.25 $\pm$ 10.24 to 380.62 $\pm$ 11.78 [84]
Gestation period (days)	145.73 $\pm$ 5.42 [66], 152.65 [84], 143.5–144.1 [88]
Post-partum heat period (days)	36.0 $\pm$ 1.254 [11], 38.75 $\pm$ 10.5 [89], 33.39 $\pm$ 1.82 [13]
Litter size (No.)	1.01 $\pm$ 0.10 to 1.06 $\pm$ 0.13 [82], 1.76 $\pm$ 0.08 [66] 2.37 $\pm$ 0.09 [83]
Kidding interval (days)	178.23 $\pm$ 0.50 to 190.2 $\pm$ 20 [82], 187.06 $\pm$ 1.09 [66] 210.6 $\pm$ 7.34 [83]

Table 1.
Reproductive performance of Black Bengal goat.

survivability is further improved by appropriate housing, nutrition, and disease prevention, all of which eventually increase total output.

8. Disease prevalence and health management

Hot and humid conditions in Bangladesh favor various diseases [90, 91]. Although BBG is resistant to various diseases, *Peste des petits ruminants* (PPR) virus is the main infectious cause of goat mortality in Bangladesh [92] and PPR is one of the major problems for the development of goat industry in Bangladesh [93]. The outbreaks of PPR caused 74.13% morbidity and 54.83% mortality in BBGs in Bangladesh [94, 95]. PPR susceptibility varied ($p < 0.01$) among breeds and the disease affected mostly (27.13%) in BBG [96]. Other studies showed that Jamunapari goat (25.13%) were more infected than BBG (16.07%) [97]. The disease observed in Rajshahi was higher during the months of December (31.68%) and January (30.34%); and lower in the months of June (9.52%) and July (11.86%) [96]. In the Chittagong region, PPR prevalence was highest in August (13.75%) and lowest in May (4.09%) [93]. In the Sylhet region, the infection rate of PPR was highest in winter (21%) and in summer (16.02%) season [97].

Caprine arthritis and encephalitis prevalence was 4.20% in different districts of Bangladesh [98]. The prevalence of contagious ecthyma was 0.80% in the northeast-ern part of Bangladesh [97].

Among all other diseases (**Table 2**), pneumonia cause high mortality and poor production of goat [104]. Age group of 1–2 years, male goat and animal with poor body condition score are at greater risk of pneumonia as well as free range (night shelter only), muddy floor with a poor drainage system, having a feeding pattern of grazing and group or community-based grazing pattern were showed susceptible to pneumonia [104].

The prevalence of parasitic infestation depends on ecology, geographical and climatic condition prevailing in Bangladesh [77]. Large flock sizes were more infected to parasitic infestation (both by ecto- and endoparasites) than the smaller ones and

Bacterial diseases	Prevalence (%)	Area and references
Pneumonia	9.43, 6.31, 37.0, 22.69	Sylhet [97], Dinajpur [99] Dhaka [100], Dhaka [101]
Mastitis	5.71, 0.98, 4.54	Sylhet [97], Dinajpur [99] Mymensingh [102]
Shipping fever	4.91	Sylhet [97]
Arthritis	3.98, 0.52	Sylhet [97], Dinajpur [99]
metritis	3.19, 1.92	Sylhet [97], Dinajpur [99]
Urinary tract infection	2.52	Sylhet [97]
Actinomycosis	2.26, 1.42	Sylhet [97], Dhaka [101]
Foot rot	1.59, 0.14	Sylhet [97], Dinajpur [99]
Tetanus	1.33, 0.37	Sylhet [97], Dinajpur [99]
Listeriosis	0.93	Sylhet [97]
Diarrhea	29.38, 19.9	Dhaka [101], Dinajpur [103]

Table 2.
Prevalence of various bacterial diseases of Black Bengal goat in Bangladesh.

this is due to direct contact, overcrowding and the unhygienic condition of goat farm [105]. Various parasitic diseases occur in BBG (**Table 3**), and among them, keratoconjunctivitis (5.71%), anaplasmosis (3.72%), fungal infection (3.59%), babesiosis (2.66%), dermatitis (2.39%) and coccidiosis (1.99%) are common [97].

A biosecurity plan must take into account all modes of transmission, including direct animal contact within a herd, contact with wild animals or other domesticated species, airborne transmission, contaminated feed or water, and visitors or vehicles that come onto the farm [111]. Advice and treatment from a veterinarian are almost absolute in preventing and controlling health problems in a herd/flock [111]. Vaccinating the herd/flock can provide some insurance against specific common diseases [111]. The only way to eradicate PPR globally is through fast outbreak investigation, notification, quick response, and immediate control measures [92]. Routine vaccination, deworming and good husbandry practices can reduce the prevalence of diseases in BBG.

Parasitic diseases	Prevalence (%)	Area/Region with references
<i>External parasite</i>		
Lice infestation	1.92	Dinajpur [99]
Scabies/Mange mite	0.51	Dinajpur [99]
Mite infestation	0.14	Dinajpur [99]
Tick infestation	0.05	Dinajpur [99]
<i>Gastrointestinal parasites</i>		
<i>Strongyloides</i> spp.	34.2, 51.74	Kurigram, Sirajganj, Pabna, Mymensingh [106], Chittagong [105]
<i>Haemonchus</i> spp.	20, 41.79, 65.63	Kurigram, Sirajganj, Pabna, Mymensingh [106], Chittagong [105], Mymensingh [107]
<i>Paramphistomum</i> spp.	21.5, 39.30	Kurigram, Sirajganj, Pabna, Mymensingh [106], Chittagong [105]
<i>Eimeria</i> spp.	9.2	Kurigram, Sirajganj, Pabna, Mymensingh [106]
<i>Fasciola gigantica</i>	8.5, 21.54	Kurigram, Sirajganj, Pabna, Mymensingh [106], Sylhet [108]
<i>Trichuris</i> spp.	8.5	Kurigram, Sirajganj, Pabna, Mymensingh [106]
<i>Trichostrongylus</i> spp.	36.32	Chittagong [105]
<i>Ectoparasites</i>		
<i>Damalinea caprae</i>	47.9, 20.8	Mymensingh [109], Gaibandha, Mymensingh [110]
<i>Linognathus stenopsis</i>	24.3, 18.4	Mymensingh [109], Gaibandha, Mymensingh [110]
<i>Haemaphysalis bispinosa</i>	20.8, 34.4	Mymensingh [109], Gaibandha, Mymensingh [110]
<i>Ctenocephalides canis</i>	9.3	Mymensingh [109]
<i>Boophilus microplus</i>	27.2	Gaibandha, Mymensingh [110]
<i>Rhipicephalus sanguineus</i>	7.2	Gaibandha, Mymensingh [110]
<i>Psoroptes cuniculi</i>	5.6	Gaibandha, Mymensingh [110]

Table 3.
Prevalence of various parasitic diseases of Black Bengal goat in Bangladesh.

9. Breeding practices of Black Bengal goat in Bangladesh

Almost 100 percent of BBG does are being bred at oestrus by natural service. Although there are some organizational goat flock in the country besides traditionally kept goats, yet artificial insemination (AI) service is completely absent in Bangladesh. There are severe shortfalls of stud bucks all over the country. Unlike cattle, goat raisers castrate almost all of their male kids at an early age for economic and social reasons. Consequently, availability of breeding bucks became squeezed. Low-class people keep bucks of unknown pedigree and use them in natural service on charge. Therefore, there remains no chance for judging the breeding soundness and fertility of the buck. Impetus toward genetic improvement has been absolutely ignored. Rather, through this system of breeding, the accumulation of inbreeding depression, along with the dissemination of various venereal and infectious diseases, is taking place [112].

The current breeding policy of Bangladesh recommends selective breeding of BBG for meat purposes in medium/low input production system. But unplanned and haphazard breeding has been in operation in Bangladesh [113]. In some cases, crossbreeding has been performed, which not only decreases the purity but also leads to the genetic dilution of BBGs which is a matter of great concern. Most of the farmers used bucks with unknown pedigree and low quality. Due to the unavailability of Black Bengal breeding bucks, the same buck has been used generation after generation, which leads to inbreeding depression that gradually decreases the overall productivity of goat [112]. The present breeding scenario indicates that artificial insemination (AI) is not yet popular for goat in Bangladesh (**Table 4**). Many factors may be responsible for this situation. The farmers are not aware of taking AI services for their heated does, unavailability of goat semen, AI technicians are not interested in AI in goats due to low price and income, poor skill of AI workers, and low conception rate for AI in

Breed	Natural mating (%)	Artificial insemination (%)	Area/Region with references
Black Bengal goat	98.41	1.59	Lalmonirhat [114]
	97.6	2.4	Barguna and Patuakhali [115]
	100	0	Dinajpur [116]
	84	16	Sirajgonj, Natore, Pabna, Bogra and Rajshahi [28]
	100	0	Mymensingh [29]
	99	1	Barguna and Patuakhali [29]
	100	0	Mymensingh [113]
	100	0	Lalmonirhat, Rangpur, Tangail, Khulna, Mymensingh [112]
	100	0	Rangpur* [117]
	85.37	14.63	Mymensingh [117]
	92.59	7.41	Narsingdi [117]

*Rangpur, Gaibanda, Dinajpur, Kurigram, Nilphamari, Bogura, Naogaon, Natore, Pabna, Sherpur, Dhaka, Brahmanbaria, Cumilla, Barishal, Patuakhali, Bhola.

Table 4.
Present scenarios of breeding practices of Black Bengal goat in Bangladesh.

goat make difficulties to popularize goat artificial insemination [118]. In a study, it was found that 65.7% of farmers found scarcity of breeding bucks, 51.4% reported scarcity of quality breed in time, and 28.5% lack of artificial insemination facilities in goat rearing [119]. In this situation, AI programs in goat should be strengthened not only to maintain purity but also to decrease the inbreeding depression and increase the productivity of BBG. Besides these, community-based planned breeding program may play an important role to reduce the scarcity of breeding bucks as well as helping for the sustainable genetic improvement of BBG [66].

10. Initiatives for the conservation of Black Bengal goat in Bangladesh

Black Bengal goat (BBG) is essential to Bangladesh's rural economy since it gives smallholder farmers a reliable source of income. Conservation of this breed is necessary to maintain its unique genetic traits, prevent dilution from crossbreeding, and foster resilience through selective breeding. The breed is a vital component of sustainable farming since it enhances food security, has cultural significance in traditional and religious activities, and works well with organic farming. Because Bangladesh recognizes the importance of preserving the breed, it has implemented both *in situ* and *ex situ* conservation strategies. Enhancing conservation through research, selective breeding, and farmer support programs is necessary to guarantee long-term agricultural sustainability and economic stability. In a study, Akhtar [103] reported that selective breeding greatly enhanced body weight, growth rates, and reproductive qualities over three generations in their study of the characterization and *in situ* improvement of BBGs in a community-driven breeding program in Bangladesh. The establishment of "Community-Based Buck Parks" in rural Bangladesh significantly improved the genetic development and conservation of BBGs [120]. Considering the significance of BBG conservation, many institutions and organizations have launched conservation and development initiatives such as Bangladesh Agricultural University, Bangladesh Livestock Research Institute (BLRI) (<https://blri.gov.bd/>), Jagorani Chakra Foundation (JCF) (<https://jcf.org.bd/>), Integrated Development Foundation (IDF) (<https://idfbd.org/>), WAVE Foundation (<https://wavefoundationbd.org/>). Moreover, the Bangladesh government, in collaboration with international organizations like the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization (FAO), has:

- Conducted research to plan and protect the future of Black Bengal goats.
- Trained farmers on modern goat-rearing techniques to adapt to changing environments (<https://www.iaea.org/>).

Overall, the goals of these institutional programs are to improve the productivity of the Black Bengal goat, preserve its genetic purity, and raise the standard of living for Bangladeshi rural populations.

11. Adaptation of modern reproductive biotechnologies for Black Bengal goat improvement

Evaluation of breeding bucks based on semen quality is important and provides the guideline for buck evaluation for reproductive performance [121]. Effects of

cryopreservation of BBGs sperm motility and fertility were critically examined. It was observed that cryopreservation had a significant effect on post-thaw sperm motility of different BBG bucks ranging from 42.86 ± 3.25 to $52.33 \pm 1.45\%$, and fertility was varied from 36.4 to 47.4% [122]. Estrus detection is critical for successful artificial insemination (AI) and controlled breeding programs. However, estrus detection of animals is laborious, time-consuming, and exposed to human error [87, 123]. Synchronization of estrus has been practiced as a labor-saving tool for those producers who want to capitalize on superior genetic material available through AI [124, 125]. Research on BBG reproductive management has demonstrated the efficacy of synthetic hormones in synchronizing estrus. One milliliter of Dinoprost®, may be used for inducing estrus of BBG under Bangladesh conditions [87]. On the other hand, 12.5 and 15.0 mg progesterone could be used for estrus synchronization in BBG [126].

Multiple ovulation and embryo transfer (MOET) technique is mainly performed to speed up the process of genetic improvement of any economic traits of the mother by increasing the selection intensity and accuracy of selection. In the MOET study, the success of the MOET technique not only depends on the superovulation rate but also on the embryo yield [127]. Researchers reported that the percentage of fertilized and transferrable embryos was 72% and 51%, respectively, in BBG. Goats treated with alfaprostol showed a noticeably higher rate of fertilization than goats treated with luprostiol [127].

Numerous studies have emphasized significant findings in the fields of *in vitro* fertilization (IVF) and embryo generation in goat reproductive science. BBGs showed seasonal differences in ovarian follicle formation, with summer-time degeneration rates being higher than winter-time rates [128]. For the formation of *in vitro* embryos, ovaries lacking a corpus luteum (CL) produce more high-quality cumulus-oocyte complexes (COCs) [129, 130]. Supplementing with 10% goat follicular fluid (GFF) significantly improved embryo development, with higher rates of cleavage and morula formation [131]. Another important factor was the quality of the embryos; A-grade zygotes produced better outcomes than B-grade ones [132]. For BBG oocyte maturation and fertilization in TCM-199 medium, a 5% concentration of bovine serum albumin (BSA) was advised [133]. It was found that semen from fresh and frozen-thawed bucks worked just as well for IVF in goat oocytes [134]. Furthermore, it was demonstrated that a 10% GFF supplement had a good impact on the production of BBG embryos [135]. Besides these, goats' right ovaries were bigger than their left, and ovaries lacking CL produced more follicles and normal COCs than ovaries with CL [136]. The slashing approach produced significantly more grade A and B oocytes, better metaphase II stage oocytes, and higher cleavage and blastocyst rates in caprine ovaries than the slicing and aspiration methods [137]. When compared to slicing, the aspiration procedure produced grade A oocytes and a far greater recovery rate during the evaluation of the ovarian state and ovum pick-up techniques in BBGs [138].

12. Socioeconomic contribution of goat farming in Bangladesh

12.1 Goat farming and rural livelihoods

In Bangladesh, BBG farming is a vital source of income for smallholder and landless farmers. It is a high-return, low-investment endeavor that offers nutrition,

employment, and income. Particularly significant is BBG rearing, which is renowned for its versatility, high rate of reproduction, and excellent meat and skin quality. By supplying meat, milk, premium skin, and organic fertilizer, BBG makes a substantial contribution to rural economies [139]. A reliable source of income in times of financial difficulty, goats provide financial security [140]. Therefore, to support this sector, the Department of Livestock Services (DLS) in Bangladesh has established Government Goat Development Farms (GGDF) in Dhaka, Sylhet, Rajshahi, Chuadanga, and Jhenaidah to conserve and expand BBG breeding and support the poor farmers.

12.2 Goat farming as a tool for poverty alleviation

Goat farming plays a significant role in poverty reduction, especially for landless farmers who depend on communal grazing areas. BBG is often called the “poor man’s cow” due to its affordability and ability to thrive on local feed resources [141]. Unlike large livestock, goats offer quick financial returns due to their short reproductive cycles and low maintenance costs [142]. As a “walking bank,” goats provide a financial buffer, easily sold in emergencies to cover household expenses [143]. Recognizing this potential, governments and NGOs have taken initiatives to promote goat rearing, especially in semi-arid and flood-prone areas where crop farming is unreliable [144]. Goats contribute to household food security and commercial markets through meat, milk, skin and manure [89, 145].

12.3 Enhancing food security through goat farming

Food security is still a big problem in Bangladesh because of the country’s fast population expansion and scarcity of arable land [146]. A sustainable supply of premium protein and vital nutrients is offered by goat rearing. Protein, vitamins B12, B6, and niacin, as well as minerals like iron and selenium, are abundant in goat meat (chevon) [147]. BBG meat is widely accepted and is in high demand, particularly for its superior quality [141]. Goat milk, which is easier to digest than cow’s milk, is a valuable nutritional resource, particularly for children and individuals with milk allergies [148]. Therefore, to increase productivity and lower malnutrition, government and non-governmental organization initiatives seek to improve goat breeding, healthcare, and nutrition [149].

12.4 Empowering women through goat farming

Since rural women are mostly responsible for caring for small animals, BBG farming is a major factor in the empowerment of women in Bangladesh. It increases their financial security and decision-making authority by giving them a separate source of income [150]. Because of their small size and ease of handling, goats are managed by women and children on homesteads or communal areas [151]. Women can directly contribute to household income through goat farming, which raises their social standing [152]. Many women are now self-sufficient and provide substantial support for their families [79]. Through improved management techniques including immunization, deworming, and supplemental feeding, BBG farming projects have assisted rural women in increasing household income by 200% [153]. Initiatives run by NGOs and the government help women move to small-scale

commercial farming by providing them with better breeding stock, microfinance, and training [154].

Findings from the project “National program on poverty reduction through goat production” showed that BBG rearing improved livelihood dramatically and food purchasing capacity increased from 20 to 28% per annum that uplifted the farmer’s social status. Moreover, health facilities, festivals and educational status acceptance were increased by 28, 26 and 19%, respectively, by BBG rearing [155]. Moreover, one recent study conducted on entrepreneurship development among women through community-based goat rearing in char areas of Bangladesh found that community-based BBG rearing helped to develop entrepreneurship among women and 70% of women in char areas opined that their overall livelihoods have been improved. The majority of the women entrepreneurs (71.61%) received benefits from BDT 10,000 to more than BDT 20,000, which facilitated them to help their husbands during a crisis moment, can give money to their children for education, and buy personal commodities. Most importantly women had some cash money in their hand to spend them freely according to their need in the family [156]. Overall, BBG rearing has helped to scale up the rural livelihoods, alleviate poverty, enhance food security and empower women in Bangladesh.

13. Conclusion

BBG farming is important for rural development in Bangladesh because it reduces poverty, ensures food security, and empowers women. BBG remains a key asset for smallholder and landless farmers due to its economic and nutritional benefits. The socioeconomic impact of goat farming will be further enhanced, boosting the livelihoods of millions of rural communities, by reinforcing government and non-governmental organization initiatives in breed improvement, improved husbandry practices, and market development. There are many challenges facing goat farmers in the country, including scarcity of pasture/feed, seasonal fluctuation and availability of feed resources, scarcity of superior buck, lack of training for better management of their goats, insufficient veterinary services, lack of credit facilities and also marketing facilities. Government and NGOs should come forward and work collaborately to mitigate the issues faced by the BBG rearers. Investment in the research and development to improve the productivity of BBG should be an approach. Thus, it is possible to boost up the goat production as well as poverty alleviation, women empowerment and employment generation in the country.

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Section 4

Genetic and Phenotypic
Innovations in Fiber
Livestock

New Insights into the Color Phenotypes and Genotypes of Huacaya Alpaca and Their Fiber's Economic and Commercial Value

Rubén Pinares

Abstract

This topic highlights color phenotypes and recent genetic findings related to pigmentation in Huacaya alpacas. The wide color diversity is the result of human selection based on fiber color. Main phenotypes of dark colors were grouped by lightness and chromaticity values: true black and dilute black (1), dark brown (2), and light brown (3). Pigmented fibers with the lowest lightness and chromaticity values tend to be true black, dilute black, or dark brown. Recent advances in molecular genetic analysis and genomic assembly (VicPac4) have identified two genes that control fiber pigmentation: *ASIP* (agouti signaling protein), which is located on chromosome 19, and *MC1R* (melanocortin 1 receptor), which is located on chromosome 21. There is a strong correlation between standard CIE $L^*a^*b^*$ fiber measurements (lightness and chromaticity values) and alpaca genotypes. Variation in dark-colored alpaca fleece is due to specific alleles at the *MC1R* and *ASIP* genes. Selecting black and dark brown Huacaya alpacas based on these alleles and genotypes improves uniform fleece production for the global textile industry. The $a^1a^1/E-$, $a^1a^2/E-$, $a^3a^3/E-$, and $a^1a^3/E-$ genotypes should be selected primarily. Fiber colorimetry combined with genotyping can improve breeding decisions and support its use as an inexpensive tool for breeders. The segregation of true black fleece can be explained by epistatic interactions between *ASIP* and *MC1R* alleles. However, the effects of other genes and the ascendance lines used are not well understood in alpaca breeding. These insights have significant economic and commercial implications because the market value of dark-colored fiber is currently low. Breeding strategies and processing fibers of natural colors are important. Improving the predictability of uniform colors through colorimetry and genotyping in selective breeding could enhance product quality, diversify textile applications, and increase the price and profitability of colored fiber for Peruvian alpaca breeders.

Keywords: gene interaction, MC1R, ASIP, haplotype, color genotype, alpaca breeding, fiber pigmentation

1. Introduction

Alpacas are widely bred in the South American highlands. Breeding alpacas has great productive and commercial potential while minimizing environmental impact, according to RAS– Responsible Alpaca Standard. Their biological characteristics, such as morphology and feeding habits, reduce their carbon footprint [1]. These characteristics also contribute to the conservation of their ecosystem, making alpacas an ideal option for sustainable livestock farming in the Andes. Peru is one of the leading producers of alpacas, with 4,533,924 animals, accounting for around 85% of the global population. The major importers of alpaca fiber and its derivatives, such as tops, yarns, fabrics, and garments, are the following countries: China (24%), Italy (19%), and the United States (16%) [2].

Since their domestication, alpacas have been selected based on fiber color, but six decades ago, selection focused on white fiber. This practice has had negative effects, causing changes in diversity and population structure. Consequently, the population of colored alpacas has decreased drastically due to the increased demand for white fiber in the textile industry. This fiber can be dyed in various shades of brown, black, and mixed colors. From an ecological viewpoint, it is necessary to implement conservation and genetic improvement plans or programs for colored alpacas at the community level in the Peruvian Andes [3–6]. Visual recording of color in herds limits its correspondence to genotype. Therefore, measurement fiber color using colorimetry based on the CIE $L^*a^*b^*$ system and determining genotype through sequencing the *MC1R* (melanocortin 1 receptor) and *ASIP* (agouti signaling protein) genes facilitate the selection of breeding stock and accelerate the genetic improvement of productive and textile characteristics in black and brown fiber.

2. Biodiversity of colored alpacas

The biodiversity of colored alpacas has been subjected to a continuous process of bleaching through the use of male breeding alpacas. Small breeders in rural communities are the ones who still keep colored alpacas in their herds, primarily for cultural reasons and food security (**Figure 1**). They maintain ancestral knowledge and implement strategies to minimize genetic erosion and safeguard their genetic diversity [6]. Additionally, public and private institutions working in the alpaca sector have led individual or collective efforts in the genetic improvement or in conservation of colored alpaca biodiversity. These efforts include national universities and research centers located in southern Peru (**Figure 2**). Likewise, the National Institute of Agricultural Research's (INIA) Quimsachata Research and Production Center has a germplasm bank for colored alpaca herds. The Pacamarca farm company manages a herd of over 695 black alpacas. Both herds are located in Peru's Puno region.

3. Genomic research in alpacas

The National Institute of Agricultural Research (INIA) in Peru created a genome library of colored alpacas to identify and preserve those in danger of extinction [7].



Figure 1.
Dark-colored Huacaya alpacas at the Livestock and Agriculture Expo Show in Marangani, Cusco.



Figure 2.
An alpaca herd from the South American Camelid Research Center (CICAS) – La Raya, belonging to the National University of San Antonio Abad del Cusco.

Subsequently, the Universidad Nacional Agraria La Molina (UNALM) developed a 76 K alpaca single-nucleotide polymorphism (SNP) microarray [8]. Recently, efforts have been made to improve knowledge of the alpaca genome, its genetics, and cytogenetics to develop molecular tools for selection and breeding. Thus, in the

reference *Vicugna pacos* genome, VicPac4 (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_048564905.1/), the *ASIP* gene is located on chromosomes 19. However, the MC1R gene is not correctly annotated in the VicPac4 assembly, and currently is encompassed within exon 2 of the *Tubulin beta-3 chain* gene), [9].

An comprehensive genetic improvement program is required to make these molecular markers, color genotyping, and the effective application of this technology available to alpaca farmers in Peru through by the Ministry of Agriculture's extension program, which utilizes genomic selection or another technology. The next step is to advance colored alpaca breeding in Peru by consolidating the mating system, artificial insemination technique, promoting its use among breeders, and establishing a nucleus of of high-merit sires. This may require purchasing males from communal farms in southern Puno that have conducted genetic selection programs for decades. Ultimately, the goal is to benefit the more than 500,000 families currently living in extreme poverty in the highlands [7] by using molecular markers to test natural colors.

4. Color measurement on the alpaca fiber

The fundamental operating principle of colorimetry is similar to human color vision perception, which occurs when photoreceptor cells in the eyes are stimulated and the brain interprets the signals. The human eye can perceive differences in a wide range of alpaca fiber colors. Different terminologies are used for shades of brown because it is subjective. For example, one person's fawn may be another person's light brown, and one person's medium brown may be another person's red-brown (**Figure 3**). The current nomenclature for alpaca colors contributes to the confusion.



Figure 3.

According to the industrial chart, up to 16 natural colors can be produced from alpaca fiber: white to black and everything in between, plus beautiful fawns and browns.

Therefore, it is recommended that a standard colorimetry system be used. Since color perception has physical and psychological components, as determined by the Commission Internationale de l'Éclairage (CIE), the factors influencing the observer must be considered when assigning a color to alpacas. The CIE $L^* a^* b^*$ system is a three-dimensional color space consisting of three axes. The L^* coordinate is represented on the vertical axis and ranges from 0 (black) to 100 (white). The a^* value indicates the red–green component of a color. Negative and positive a^* values, ranging from -100 to +100, indicate green and red, respectively. The b^* axis represents the yellow–blue component. Negative and positive b^* values, ranging from -100 to +100, indicate blue and yellow, respectively (**Figure 4**) [10, 11].

L^* , a^* , and b^* values were recorded using the same technique of holding the sample against the machine's aperture. The distance from the central axis represents chroma (C^*), or a color's saturation. The angle on the chromaticity axes represents the hue (h). These values were calculated based on the following formulas $C^* = \sqrt{a^{*2} + b^{*2}}$, and $h = \tan^{-1}(\frac{b^*}{a^*})$ [4, 11, 12]. Colorimetry analysis is a standardized tool that is widely used for measuring alpaca fiber [4, 10, 13–16].

Objective color quantification can be obtained using colorimetric devices, such as a tristimulus colorimeter or a spectrophotometer. A common choice is the Chroma Meter CR series (Konica Minolta, Tokyo, Japan). Before using the colorimeter, calibrate the device using the standard calibration provided by the manufacturer.

Figure 5 illustrates the primary components of a colorimeter: an illuminant, colored filters that imitate the spectral sensitivity of cones in the human eye, and a processor that compensates for the Standard observer. A colorimeter typically includes an illuminant, a lens, filters, and a computer analyzer. The illuminant, represented by the light source, emits specific wavelengths onto a sample. The sample absorbs these wavelengths and reflects light. The colorimeter captures the

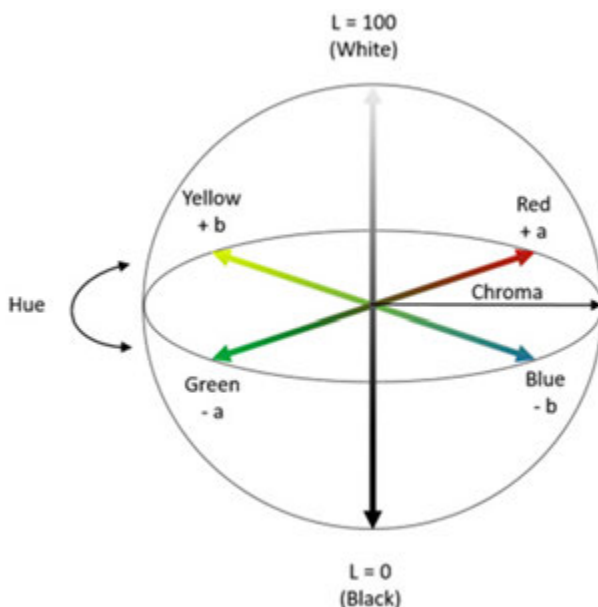


Figure 4.
 Color space diagram: L^* , a^* , and b^* values are useful for measuring the color of alpacas [10, 11].

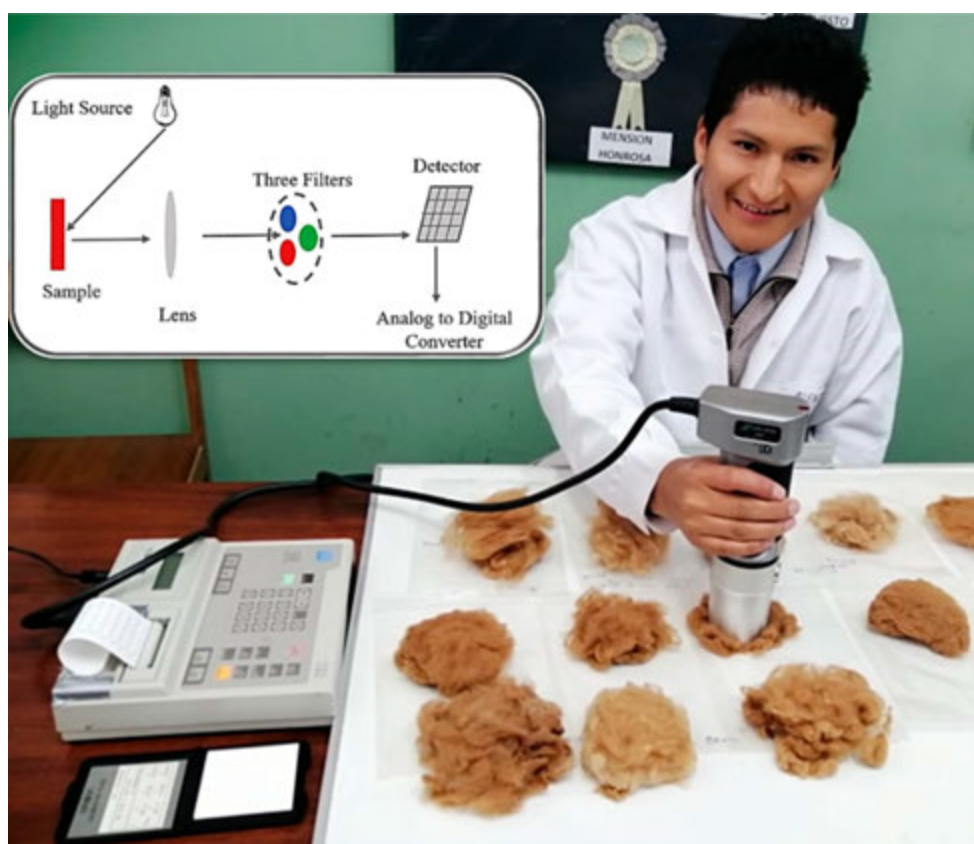


Figure 5.

The operating principle of the Chroma Meter CR-210 colorimeter at the fiber laboratory.

reflected light and filters it through a trichromatic filter of red, green, and blue chromas. The user can personalize downstream processing of the data by selecting parameters such as the observer color-matching function and the illuminant spectral setting.

Research based on the CIE $L^* a^* b^*$ system indicated that it is possible to predict the white and black colors in alpacas using lightness, the red/green axis, and the yellow/blue axis of three-dimensional space color. This method could be helpful for assigning the right natural color to an alpaca fiber fleece for genetic improvement and textile manufacture. Addressing the improvement of textile characteristics is currently required in the natural color production of alpaca fiber [15]. Lightness measures the degree of whiteness of the fibers. In white fibers, lightness is high, at about 84.52; in light brown fibers, it decreases to about 30.85; and in black fibers, it decreases to about 18.68 (**Table 2**). Both lightness (**Figure 6**) and chromaticity (**Figure 7**) decrease slightly from white or light brown to true or dilute black.

Spearman's rank correlations for visual alpaca phenotypes with both lightness ($r_s = 0.85$) and chromaticity ($r_s = 0.92$) were strongly positive ($p < 0.0001$). Additionally, when considering pigmented fibers, both colorimeter values were positively correlated ($r = 0.81$; $p < 0.0001$).

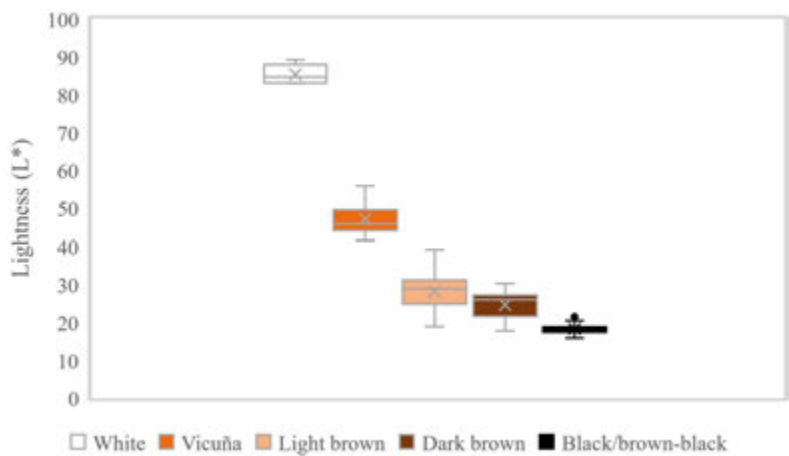


Figure 6.
Box-and-whisker plot of lightness values in colored fibers.

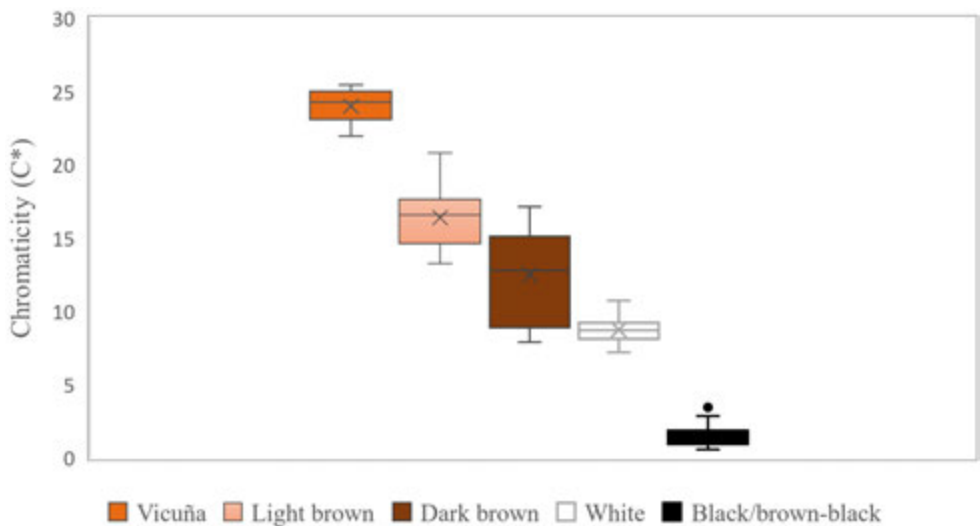


Figure 7.
Box-and-whisker plot of chromaticity values in colored fibers.

5. Alpaca color genetic

The pigmentation of fiber and skin is controlled by several genes, including *ASIP*, *MC1R*, *TYR*, and *TYRP1*, which are activated at different times and in different locations. Sometimes, these genes act simultaneously when interacting with one another. Melanogenesis is a synthetically complex pathway regulated by the *ASIP* and *MC1R* genes. This pathway involves a series of chemical, enzymatic, and non-enzymatic reactions that produce two types of melanin: eumelanin and pheomelanin. Thus, *MC1R* and *ASIP* are crucial for determining melanin type and fiber color. When

α -MSH binds to MC1R, it stimulates eumelanin production, responsible for true and dilute black. Conversely, functional ASIP acts as an MC1R antagonist, increasing pheomelanin production and decreasing eumelanin synthesis (**Figure 8a**) by blocking the MC1R signaling pathway [10, 17]. Color patterns on fleece or in individual fibers are related to ASIP regulation (**Figure 8b**). Nevertheless, the molecular mechanisms underlying color variation in alpacas remain poorly understood.

The ASIP gene in alpacas, also known as the agouti gene, has a complex structure that includes three coding exons (named 2, 3, and 4) and large introns (**Figure 9**).

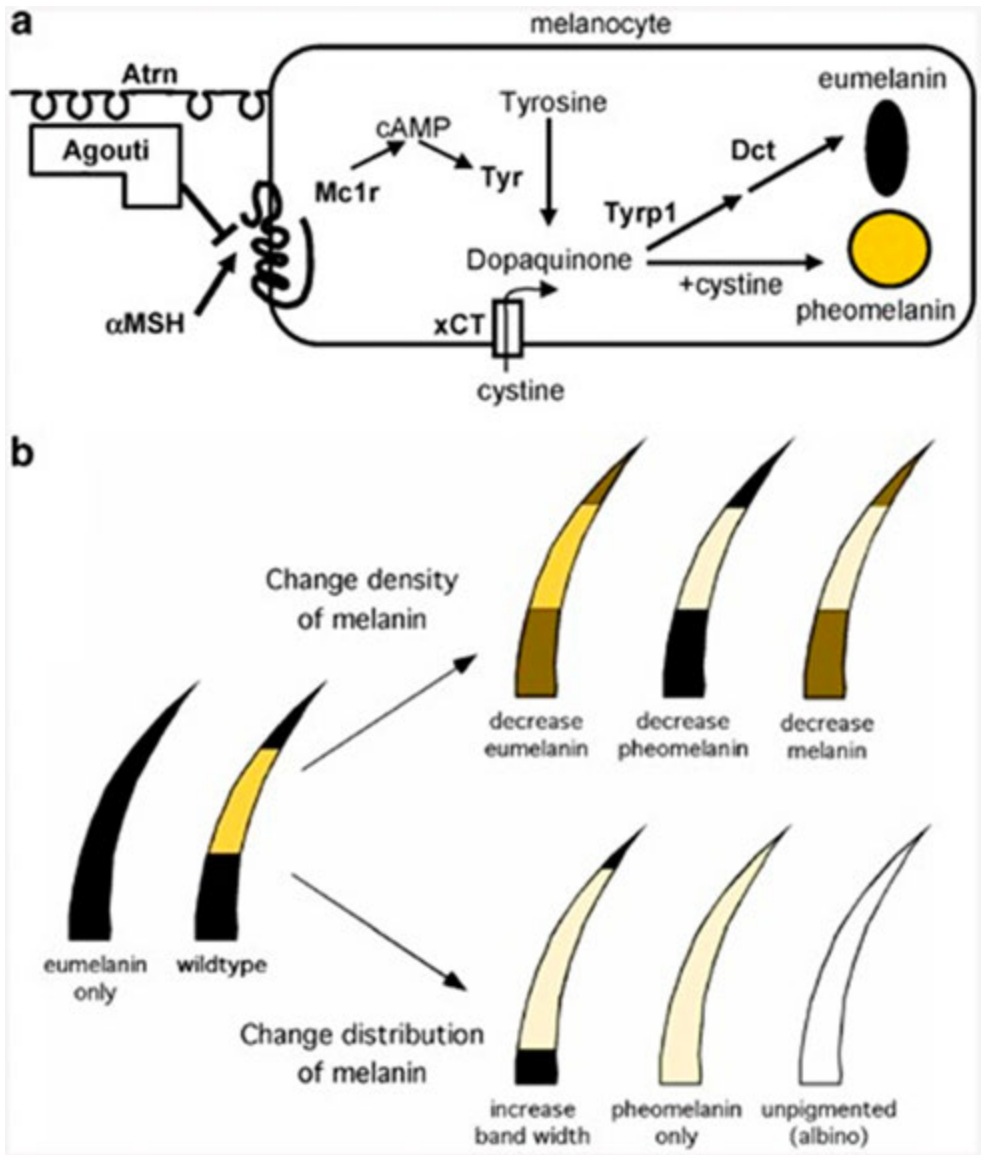


Figure 8. Genetic pathways regulating mammalian melanogenesis (a) and phenotypic effects on individual fiber pigmentation and patterns. (b) [17].



Figure 9.

The structure of the alpaca *ASIP* gene is shown, including coding exons 2, 3, and 4. Polymorphisms are shown in the regions where they occur. Non-synonymous polymorphisms are in bold [18].

Two non-synonymous polymorphisms (c.292C > T and c.353 G > A) and a 57-bp deletion (c.325_381del) were identified in exon 4 of the *ASIP* gene [18]. Based on haplotype analysis, three loss-of-function alleles (a^1 , a^2 , and a^3) were identified. These alleles were commonly present in true or dilute blacks, dark brown, and light brown alpacas. The wild allele (A^+) was present in 50% of white alpacas, 18.18% of light brown alpacas, and 100% of vicuñas in homozygosis.

The predicted lengths of *ASIP* exon 2 (160 bp) and exon 4 (177 or 120 bp) were confirmed by sequencing. Considering the unchanged size of exon 3 (65 bp), the non-deleted CDS was 402 bp and translated to 133 amino acids. However, the deleted CDS was 345 bp and translated to 114 amino acids (**Figure 9**). *MC1R* gene has a 954-bp coding sequence with c.224_227del and nine polymorphisms. Five of these polymorphisms were non-synonymous (c.82A > G, c.259 G > A, c.376 G > A, c.587 T > C, and c.901C > T), resulting in the amino acid changes p.T28A, p.M87V, p.G126S, p.F196S, and p.R301C, and four were synonymous (c.126C > T, c.354C > T, c.618 G > A, and c.933A > G). The functional protein synthesizes 317 amino acids, whereas the nonfunctional protein synthesizes only 84 (**Figure 10**). Based on haplotype analysis, the dominant (E), recessive (e), and wild-type (E^+) alleles of *MC1R* or extension gene were described (**Table 1**). The a^1 , a^2 , and a^3 alleles of the *ASIP* gene, in combination with the E allele of the *MC1R* gene, define black and brown-black colors. Brown alpacas have greater allelic variability in the *MC1R* and *ASIP* genes, resulting in heterozygous genotypes and great phenotypic diversity.

The color of an alpaca is controlled by its genes. By evaluating the color objectively, you can usually determine which genes and which variants are responsible for the color you see. Including information about an animal's parents and offspring increases the success of predicting color. DNA tests for these genes can determine an animal's genetic color potential, allowing breeders to plan matings to produce or avoid a particular color. We suggest using names that reflect alpaca colors' genetics. This will lead to more effective color assignment, precise records, and higher predictability in breeding outcomes [19]. Therefore, color varieties reflect their genetic basis. The *ASIP* and *MC1R* sequences are more conserved, or homogeneous, showing less genetic variation in wild vicuñas than in domestic alpacas. This aligns with the domestication process, which involved selecting diverse color phenotypes in alpacas [4, 18, 20]. This is confirmed at the genomic level. There is a relationship between the described genotypes and the

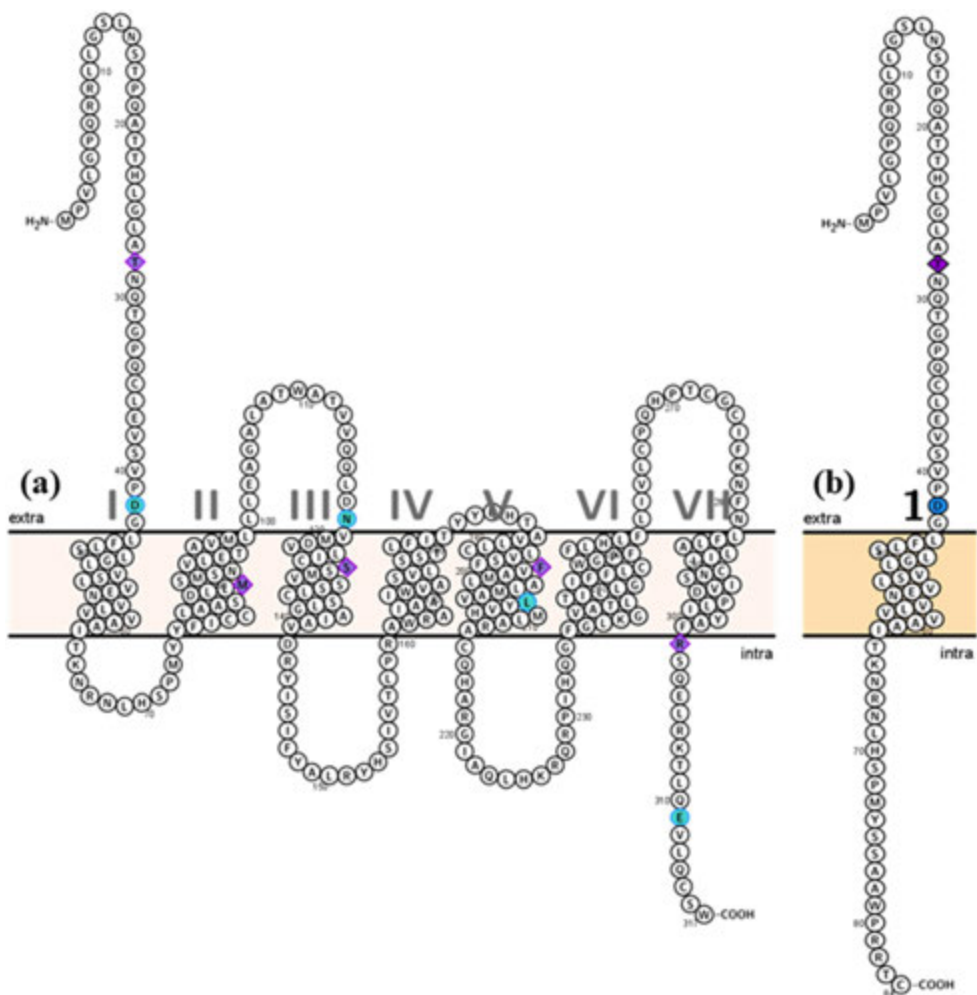


Figure 10.
The predicted structure of the alpaca MC1R protein shows both normal (a) and frameshift (b) amino acid sequences [4, 18].

phenotype categories. In addition, the lightness and chromaticity values were associated with the *ASIP* and *MC1R* genotypes [20]. True or dilute black alpacas typically have the E allele in homozygosity and/or heterozygosity, combined with the recessive a^1 , a^2 , and a^3 alleles (**Figure 11**). Thus, the interaction between the *ASIP* and *MC1R* genotypes proportionally modulates eumelanin and pheomelanin synthesis in colored phenotypes [16, 18, 20].

It is not uncommon for animals to have the blackest possible genotype and appear brown. Despite being brown, these alpacas can produce black offspring and are effective in a black breeding program. A true black phenotype is not uncommon in this genotype, just as it is not uncommon for them to have the blackest genotype (EE aa) and still appear brown. However, brown alpacas are more often found in the Ee aa genotype than in the EE aa genotype (**Table 2**). With this genotype, it is certain that they will pass a recessive *ASIP* allele (a) to their offspring, but they may pass

MC1R haplotypes		Counts	BL/BB	DB	LB	WH	VV
[H1]	AAATC	94	38(11;27)	16(7;9)	19(3;16)	0	0
[H2]	AGGTC	24	10	6	6	2	0
[H3]	GGGTT	24	10	7	4	2(1;1)	0
[H4]	GGGTC	37	3	1	4	6(5;1)	9
[H5]	GAGTT	01	1	0	0	0	0
[H6]	GGGCC	10	2	0	8	0	0
[H7]	GGATT	01	1	0	0	0	0
[H8]	AGGTT	01	0	1	0	0	0
ASIP haplotypes							
[H1]	TGN	23	16(2;14)	3	2	0	0
[H2]	C–D	34	20(2;18)	6	4	3	0
[H3]	CAN	74	26(11;15)	12(3;9)	16(2;14)	1	0
[H4]	CGN	65	2	11(1;10)	17(4;13)	8(4;4)	9

MC1R haplotypes consist of five amino acid substitution polymorphisms: c.82 G > A (p.A28T), c.259 G > A (p.V87M), c.376 G > A (p.S126G), c.587 T > C (p.F196S), and c.901 C > T (p.R301C). ASIP haplotypes consist of three non-synonymous polymorphisms: c.292C > T, c.353 G > A, and c.325_381del. D: deletion; N: no deletion. n(a;b): number of animals; a: homozygosity; b: heterozygosity. Colors: true black/brown black (BL/BB), dark brown (DB), light brown (LB), white (WH), and vicuña (VV) [4, 18].

Table 1.
Specific haplotypes of MC1R and ASIP genes in colored alpacas and vicuñas.



Figure 11.
Fleece color phenotypes and their corresponding MC1R and ASIP genotype combinations.

either an active or inactive *MC1R* copy. Pairing two black alpacas with this genotype produces fawn crias if they inherit the recessive *MC1R* (e) allele from both parents. An “EE aa” animal bred to another “EE aa” animal will never produce a light fawn; for instance, while pairing two phenotypically black, “Ee aa” animals will produce a light fawn 25% of the time [16, 21].

6. Economic and commercial value of alpaca fiber

In 2022, Peru produced 4,422 tons of fiber, about 90% of which was intended for export. The total export value was over USD 187 million [22].

Phenotype	Confidence intervals of lightness	Confidence intervals of chromaticity	ASIP and MC1R genotypes
Vicuña (5)	45.8–49.7	23.6–25.1	A ⁺ A ⁺ /E ⁺ E ⁺
White (4)	83.0–86.1	7.5–9.8	A ⁺ a ¹ /E ⁺ E ⁺
Light brown (3)	29.3–32.7	15.8–17.4	A ⁺ a ³ /Ee A ⁺ -/-e
Dark brown (2)	25.8–28.9	9.9–12.9	A ⁺ a ³ /Ee- a ³ -/E-
True black and diluted black (1)	16.0–22.0	0.9–3.2	a ¹ a ¹ /E- a ² a ² /E- a ¹ a ² /E- a ¹ a ³ /E-

Table 2.
Phenotypic and genotypic assignment for V. vicugna mensalis and colored huacaya alpaca [20].

However, these estimates fluctuate depending on final consumer demand from year to year. Conversely, the price of fiber and the payments received by owners in remote Peruvian communities are far below the prices charged throughout the industrial chain. Additionally, the price varies by color; dark fiber sells for about 60% less than white fiber. Dark-colored raw fiber is less valuable. This could be due to a lack of genetic improvement or lower production. It sells for a low price of USD 2.26 per pound in the textile market (**Figure 12**), whereas white fiber sells for a high price, more than USD 5.92 per pound [6, 22]. Recently, due to natural and ecological consumer demand, colored fiber has become more attractive. Therefore, the population of colored alpaca herds should increase through conservation strategies implemented by private businesses or producers from communities. Once the standard phenotypes and genotypes have been registered, it is hoped that mating can be improved and colored herds can be consolidated. This will stabilize the population of naturally colored alpacas and satisfy niche markets by producing larger volumes of uniform colors in the future.

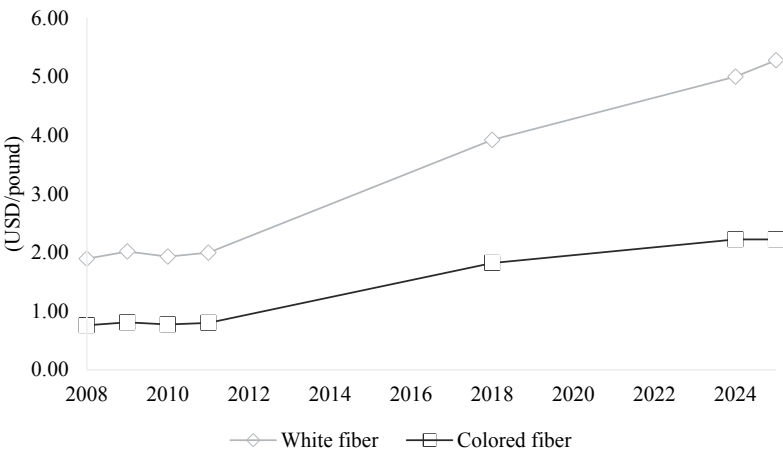


Figure 12.
Annual trends for the approximate price of raw alpaca fiber (in USD per pound) based on local marketing through intermediary collections [3, 6, 22].

7. Conclusion(s)

Our findings show that measuring alpaca fiber color based on the CIE L*a*b* system is a valuable tool for precisely describing the dark color variation. The relationship between phenotypes and their underlying genotypes provides breeders with valuable insights. Alpaca fleece color is determined by the epistatic interaction, or synergy, at least of two key genes: *ASIP* and *MC1R*. The specific alleles of these genes control the production and distribution of melanin types that produce a wide range of dark color phenotypes, including true black, dilute black, dark brown, and light brown. Our results provide a molecular basis for further research on alpaca fleece color mechanisms. The ability of a single phenotypic color to be produced by more than one genotype underscores the necessity of moving beyond simple visual selection. Combining colorimetry (CIE L*a*b* measurements) with genotyping is a powerful tool for improving the selective breeding of Huacaya alpacas. This approach enhances the predictability of a uniform fleece color, a critical challenge for the textile industry. Consistently producing high-quality, uniformly colored fiber allows native Peruvian alpaca breeders to increase their products' market value, diversify textile applications, and ultimately boost profitability.

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Conflict of interest

The author declares no conflict of interest.

Notes/thanks/other declarations

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Section 5

Alternative Feed Resources
and Sustainable Poultry
Production

Chapter 6

The Potential Role of *Azolla* as an Alternative Protein Source in Sustainable Poultry Farming: A SWOT Analysis

Soumaya Boukrouh

Abstract

The increasing worldwide demand for sustainable livestock production has necessitated the exploration of alternative feedstuffs to reduce reliance on conventional feed resources, such as soybean meal and alfalfa. *Azolla*, an aquatic fern with a unique nutritional profile, has emerged as a promising candidate. In this chapter, the author reviews the potential use of *Azolla spp.* as an alternative protein source for poultry worldwide, focusing on its nutrient composition, benefits, limitations, and potential for sustainable agricultural practices. *Azolla* offers high protein content, essential amino acids, and significant mineral levels, while requiring minimal resources for cultivation. Its rapid growth, nitrogen-fixing ability, and environmental adaptability make it a versatile feed option. However, challenges such as invasive potential, high water content, and anti-nutritional factors necessitate the development of effective processing and management strategies, such as drying or fermentation. Additionally, in this chapter, a SWOT analysis reveals its strengths and potential, but further research is needed for effective integration. Collaborative efforts from stakeholders are essential to fully realize *Azolla's* benefits in sustainable poultry systems, particularly in regions facing feed shortages.

1. Introduction

Recently, the demand for food, especially animal-based proteins, has been escalating [1]. However, conventional feed and food production face several challenges, such as severe pressure on natural resources, rangeland degradation, drought, and the high cost of concentrates [2, 3]. To address these concerns, animal production is either oriented toward climate-friendly species such as camels [4, 5] or focused on innovative, sustainable, eco-friendly, cost-effective, and nutritionally balanced solutions. Among these strategies, the integration of alternative feed resources, such as insect-based meals, food waste, algae, industrial byproducts, and drought and salinity-resistant fodder crops, has demonstrated significant potential for mitigating reliance on traditional feed sources [6–8]. These alternative feedstuffs have shown a positive impact on ruminants [9–12]. In poultry, alternative feed

resources have also been used, but with incorporation levels adapted to the requirements of monogastric animals. However, ensuring food security remains critical, necessitating sustainable strategies that minimize reliance on arable land for feed production, especially in land-degraded areas.

Among the emerging candidates, *Azolla*, a small floating macrophyte, has recently emerged as a promising alternative protein source for livestock nutrition. This aquatic fern belongs to the Salviniaceae family and comprises seven to nine species that are recognized worldwide, spanning temperate to tropical regions. These species belong to two subgenera: the *Euazolla* subgenus, which includes *A. microphylla*, *A. filiculoides*, *A. rubra*, *A. caroliniana*, and *A. mexicana*, and the *Rhizosperma* subgenus, which includes *A. nilotica*, *A. pinnata*, and *A. imbricata* [13, 14]. *Azolla* is remarkable for its potential rapid growth, mainly due to its symbiotic association with the nitrogen-fixing cyanobacterium *Anabaena azollae* [15]. *Azolla* provides a protective environment with fixed carbon (CO₂) in specialized leaf cavities, which enables cyanobacteria to supply nitrogen to themselves and their host [16]. Nitrogen fixation, coupled with minimal resource requirements for cultivation, makes *Azolla* a protein-rich candidate with a protein content of 30% in several species [17, 18]. With essential amino acids, minerals, and vitamins, *Azolla* offers a versatile and eco-friendly feed option for various species [19–24]. Besides its significant promise as a feed for livestock, the symbiotic association exhibited by *Azolla* has given it considerable agricultural importance as a bio-fertilizer, biofuel, and bioremediator in wastewater treatment [25].

The objective of this study was to highlight the characteristics of *Azolla* that justify its introduction as an alternative to the protein resources typically used in Mediterranean countries, such as alfalfa and soybean meal, with a focus on its impact on livestock and poultry nutrient digestibility, feed efficiency, growth performance, health, and animal product quality. This chapter also focuses on the weaknesses, threats, and solutions to overcome them for sustainable production and use. A Strength, Weakness, Opportunity, and Threat (SWOT) analysis [26] was implemented as a structured methodology to achieve the previously mentioned purpose.

2. SWOT analysis

The following sections of this study present a thorough examination of the benefits and challenges associated with *Azolla* use as a protein feed resource. This evaluation is based on a four-quadrant analytical approach known as SWOT analysis, which examines the strengths, weaknesses, opportunities, and threats related to *Azolla* usage [26].

2.1 Strengths

Azolla species are aquatic ferns characterized by rapid growth rates, and some *Azolla* species, such as *A. filiculoides*, are reported to double their biomass in 2.1 days [27], which leads to high biomass productivity compared to some fodder species, allowing for regular harvesting, consistent supply, and ensuring that year-round production can be sustained. Concerning its use in the zootechnical field, *Azolla* is primarily a rich protein resource (up to 30%) with essential vitamins, minerals, and amino acids, which makes it a nutritious feed option for livestock. *A. microphylla* and *A. pinnata* had the highest protein content, ranging from 17.3% to 36.28% (Table 1). Other species, such as *A. filiculoides*,

	<i>A. pinnata</i> [31, 32, 20, 33, 34, 35, 36, 37, 28, 38, 18, 39, 40, 41, 42, 43]	<i>A. caroliniana</i> [32, 44, 33, 36, 45]	<i>A. microphylla</i> [17, 46, 29, 36, 47, 48]	<i>A. filiculoides</i> [47, 50, 36, 42]	<i>A. mexicana</i> [36]	<i>A. rubra</i> [36]
Nutritive value (% DM)						
Moisture	90.87–95.03	95.5	92.33–95.72	93.2	95.9	93.5
Dry matter	4.97–93.00	4.48–89.00	6.94	6.84	4.1	6.5
Protein	17.18–32.05	18.6–23.59	17.3–36.28	16.50–27.94	22.7	23.1
Crude fiber	7.93–32.10	14.03	13.29–16.61	13.60	–	–
Ether extract	1.51–12.37	2.98–4.1	2.71–3.9	3.30–5.05	4.65	2.9
Ash	13.98–31.26	18.2–40.2	13.35–40.4	15.54–30.80	24.4	33.6
Nitrogen free extract (NFE)	18.06–48.62	37.52	44.7	41.90	–	–
Neutral detergent fiber (NDF)	35.18–60.11	42.14–51.1	29.2–50.98	39.16–42.00	42.3	40.3
Acid detergent fiber (ADF)	21.51–58.2	36.03–50.3	31.34–38.00	26.58–46.10	58.2	54.2
Acid detergent fiber (ADL)	7.00–28.24	–	14.45–15.17	–	–	–
Cellulose	12.15–36.7	16.2	–	15.19	–	–
Hemicellulose	7.92–14.00	–	12.43	–	–	–
Macro minerals (% DM)						
Phosphorus (P)	0.25–1.29	0.26–0.96	0.65	0.49–0.73	1.03	0.72
Potassium (K)	0.56–1.74	1.27–5.18	–	2.01	–	–
Calcium (Ca)	0.58–1.56	0.43–2.32	1.67	0.97–1.04	1.80	1.43
Magnesium (Mg)	0.35–1.42	0.26–0.35	–	–	–	–
Sodium (Na)	0.9–1.5	1.35	–	1.53	–	–
Micro minerals (ppm)						
Copper (Cu)	17.15–34.19	18.47–27.13	47.84–47.95	28.05–34.43	30.91	29.82

	<i>A. pinnata</i> [31, 32, 20, 33, 34, 35, 36, 37, 28, 38, 18, 39, 40, 41, 42, 43]	<i>A. caroliniana</i> [32, 44, 33, 36, 45]	<i>A. microphylla</i> [17, 46, 29, 36, 47, 48]	<i>A. filiculoides</i> [47, 50, 36, 42]	<i>A. mexicana</i> [36]	<i>A. rubra</i> [36]
Iron (Fe)	14.04–39.00	0.39–14.10	1,327–13,276	1,000–8,907	5,623	8,916
Zinc (Zn)	1.56–87.59	54.38–67.62	54.71	29.22–54.70	51.73	33.92
Manganese (Mn)	7.96 – 760	378.33–482.30	313.19–313.23	288.13–771.00	350.71	281.82
Trace minerals (ppm)						
Cobalt (Co)	3.12–16.74	3.42	–	4.60	–	–
Nickel (Ni)	6.51	23.32	23.57	15.26	13.62	12.79
Chromium (Cr)	8.11	5.95	–	–	–	–
Cadmium (Cd)	0.08	0.08	–	–	–	–
Molybdenum (Mo)	0.65	0.74	–	7.00	–	–
Lead (Pb)	1.56	2.35	–	25.00	–	–
Amino acids (g/100 g protein)						
Alanine	2.32–7.5	7.34	–	2.26–6.45	–	–
Arginine	5.37–11.55	5.66–6.24	–	6.62–16.12	–	–
Aspartate	9.19–11.25	10.99	–	9.39–10.92	–	–
Glutamate	12.6–31.3	14.46	–	12.72–22.00	–	–
Glycine	4.60–5.79	5.84	–	5.72–6.18	–	–
Histidine	1.49–2.59	1.57–2.55	–	2.31	–	–
Isoleucine	3.46–6.25	3.45–5.19	–	5.16–5.38	–	–
Leucine	7.71–10.00	8.22–9.30	–	8.76–9.43	–	–
Lysine	4.28–6.10	4.39–5.63	–	6.45–7.00	–	–
Methionine	1.14–5.87	1.07–1.29	–	1.88–5.96	–	–

	<i>A. pinnata</i> [31, 32, 20, 33, 34, 35, 36, 37, 28, 38, 18, 39, 40, 41, 42, 43]	<i>A. caroliniana</i> [32, 44, 33, 36, 45]	<i>A. microphylla</i> [17, 46, 29, 36, 47, 48]	<i>A. filiculoides</i> [47, 50, 36, 42]	<i>A. mexicana</i> [36]	<i>A. rubra</i> [36]
Phenylalanine	3.87–6.25	5.86	–	4.41–5.64	–	–
Serine	4.21–5.63	5.35	–	4.10–4.60	–	–
Threonine	3.22–5.63	4.37–5.29	–	4.40–5.12	–	–
Tyrosine	3.18–5.63	4.02	–	1.59–4.10	–	–
Valine	3.77–6.88	3.76–6.73	–	5.98–6.75	–	–
Tryptophane	1.41–5.48	1.36–5.84	–	2.01	–	–
Cistine	0.09–1.60	0.16	–	2.26	–	–
Proline	4.43–4.90	4.2	–	0.43–4.48	–	–

Table 1.
 Nutritional value of Azolla species.

A. caroliniana, *A. mexicana*, and *A. rubra*, have relatively moderate protein content (18.6%–27.94%). Moreover, Parashuramulu et al. [28] reported in vitro improved in vitro crude protein (CP) digestibility in *A. pinnata*-containing diets, which was attributed to the higher degradability of true soluble protein (B1) (42.56% DM of CP with approximately 100% intestinal degradability), showing its potential as a protein supplement. Ether extract values, representing fat content, are generally low across species, with *A. pinnata* showing the widest range (1.51% – 12.37%). Regarding fibers, *A. pinnata* showed the highest neutral detergent fiber (NDF) (35.18%–60.11%) and acid detergent fiber (ADF) (21.51%–58.2%) contents, suggesting a higher structural carbohydrate content, which may limit digestibility. *A. microphylla* was characterized by variability in NDF (29.2%–50.98%). However, an in vitro study by Kumar et al. [29] and Boukrouh et al. [30] that the organic and dry matter digestibility were higher (83.15% and 84.03%, respectively; [29]), indicating a decreased amount of undigested excreted material, leading to minimized waste and efficient nutrient utilization by animals, which can decrease the pressure on land and water resources. Livestock farming is a significant source of methane, a greenhouse gas with strong warming effects on the atmosphere. For *A. microphylla*, moderate total gas production (78.49 ml/g DM and 483.36 ml/g DDM) and methane production (11.96 ml/g DM and 14.39 ml/g DDM) suggest that this plant could lower the contribution of livestock to air pollution compared to conventional feed.

A. caroliniana and *A. microphylla* had a mineral content of 40%, indicating a rich mineral profile. Indeed, *A. microphylla* also showed high levels of calcium (1.67% DM), Cu (47.84–47.95 ppm), and iron (1,327–13,276 ppm). However, for all other species, the mineral profiles differed, suggesting that their combination could provide a more balanced intake of essential mineral nutrients. The phosphorus content was the highest in *A. mexicana* (1.03%) and was relatively consistent across other species. *A. caroliniana* had the widest range (1.27%–5.18%) in potassium. Except for *A. caroliniana*, all *Azolla* species were characterized by higher iron content (14–13,276 ppm). Zinc levels were the highest in *A. microphylla* (1,327–13,276 ppm). Regarding amino acids, lysine, which is critical for protein synthesis, was found in high amounts in *A. filiculoides* (6.45%–7.00%). *A. filiculoides* also has notable methionine levels (1.88%–5.96%), which are essential for metabolic functions. Other amino acids, such as alanine, glutamate, and arginine, were also higher in *A. pinnata* and *A. filiculoides*, emphasizing their superior protein quality.

The data presented in **Tables 2–4** show the diverse effects of *Azolla*, particularly *A. pinnata* and *A. microphylla*, on poultry nutrition across various species. Moderate *Azolla* inclusion (3–6%) in quail diets showed either improved or no significant impact on DMI, FCR, or egg production [22, 51–54]. *Azolla* up to 7% in the diet also increased nitrogen, phosphorus, and calcium retention but had no significant impact on blood parameters [22]. Quails showed increased egg weight and shell thickness with up to 10% *Azolla* in their diet [51]. In poultry, *Azolla* spp., especially dried *A. pinnata*, have shown contrasting results regarding the FCR. Feeding *Azolla* up to 15% either had no impact [55, 56], increased [57], or decreased [58] FCR. Generally, below 15%, *Azolla* improved or had no impact on FCR, except in one study that reported an increase in FCR with 5% fresh *Azolla* [23]. However, BW gain and FBW were reduced with this incorporation level [56–59]. Generally, the improvement at lower levels is likely attributable to the high protein content and rich nutrient profile of *Azolla*, which includes vitamins, minerals, and essential amino acids. These characteristics enhance the protein utilization and metabolic efficiency of poultry.

Species	Country	Processing	Duration	Incorporation rates	Main findings	Rec. levels	References
<i>A. pinnata</i>	Nigeria	-	60 days	4, 6, 8, and 10%	4, 6, 8, and 10% rates reduced hen hay and hen house production and increased egg weight, shell weight, and shell thickness. <i>Azolla</i> had no significant effect on final body weight (FBW), feed conversion ratio (FCR), daily water intake, and feed cost per gram of egg.	10%	[51]
<i>A. pinnata</i> meal	India	Shade dried	42 days	3 and 6%	3% rate increased giblet percentage and wings weight, while a 6% rate had no significant effect.	3%	[54]
<i>A. pinnata</i>	India	Dried	35 days	2.5, 5, 7.5%	2.5 and 7.5% inclusion levels increased nitrogen (N) and phosphorus (P) retention. The 7.5% inclusion level increased BW gain (BWG) metabolizable dry matter (DM), and calcium (Ca) retention. The three diets had no impact on feed intake (FI), FCR, feed cost, or BWG.	7.5%	[22]
<i>A. pinnata</i>	India	Dried	42 days	2.5, 5, and 7.5%	5% inclusion level increased chicks' BW, cumulative BWG, and cumulative feed conversion efficiency (FCE) compared to 7.5% chicks but did not differ from Co and 2.5% chicks. 5% chicks were more economical than Co, 2.5, and 7.5% chicks, resulting in higher savings over the control compared to 2.5% and 7.5%. The livability in the <i>Azolla</i> -fed groups was very high.	5%	[23]
<i>Rec. recommended</i>							

Table 2.
Research concerning the use of Azolla spp. in quails.

Species	Country	Processing	Duration	Incorporation rates	Main findings	Rec. levels	References
<i>A. pinnata</i>	India	Fresh	42 days	5%	<i>Azolla</i> increased FI and FCR and reduced FBW.	5%	[63]
<i>A. pinnata</i>	Egypt	Sun-dried leaf meal	37 days	5 and 10%	5 and 10% incorporation levels decreased cooking loss and increased breast redness (a^*). No significant effect was observed on the chemical composition or sensory properties of thigh meat.	5%	[64]
<i>A. pinnata</i>	India	Dried	42 days	2.5, 5, 7.5, and 10%	2.5 and 5% <i>Azolla</i> levels in the diet decreased FCR. 2.5, 5, and 7.5% levels increased the performance index (PI). 2.5 and 10% levels increased the protein efficiency ratio (PER). The BWG was higher at 7.5%, which was comparable to 5% and 10%. No significant impact was reported on DMD and nitrogen balance.	10%	[65]
<i>A. pinnata</i>	Ethiopia	Dried	-	2.5, 5, and 7.5%	2.5 and 7.5% decreased in FBW, total BWG, slaughter weight, and dressed carcass, and 5% decreased in FCR. FI decreased with 2.5, 5, and 7.5%. The highest net return was obtained from chickens fed on 5%.	5%	[60]
<i>A. pinnata</i>	India	Dried	42 days	2.5, 5, 7.5%, and 10%	2.5, 5, and 10% inclusion levels improved FBW, BWG, average daily gain (ADG), and protein intake (PI). Co and 2.5% inclusion levels reduced (FCR, while Co and 10% inclusion levels increased PER.	7.5%	[65]
<i>A. pinnata</i>	Japan	Sun-dried	35 days	5 and 7%	5%, followed by 7% inclusion levels, showed the highest net profit, high-density lipoprotein (HDL), and the lowest triglycerides (TG) and total cholesterol.	5%	[61]
<i>A. pinnata</i>	India	Sun-dried	35 days	3.5, 4.5, and 5.5%	3.5%, 4.5%, and 5.5% had higher FI, while no significant difference was observed in mean WG and FCR.	5.5%	[66]
<i>A. pinnata</i>	India	Fresh	98 days	<i>Ad libitum</i>	<i>Azolla</i> improved BWG and decreased mortality.	<i>Ad lib.</i>	[67]

Species	Country	Processing	Duration	Incorporation rates	Main findings	Rec. levels	References
<i>A pinnata</i>	India	Dried powder	42 days	4.5 and 5.5%	Feeding <i>Azolla</i> meal did not affect weekly BWG. A 5.5% inclusion level increased the mercaptoethanol-sensitive (IgM) antibody titer (log 2) and total immunoglobulins, while a 4.5% inclusion level had no significant impact. The 4.5% inclusion level increased the dressing percentage; however, there were no significant differences in the other carcass traits.	5.5%	[68]
<i>A pinnata</i>	Iraq	Dried powder	42 days	5, 10, 15, and 20%	5, 10, 15, and 20% increased the concentrations of globulin, HDL, total protein, glutathione peroxidase, and thyroid hormones.	20%	[69]
<i>A pinnata</i>	India	Sun-dried	42 days	1.5, 3, and 4.5%	No significant effect on FBW, FI, BW gain, feed efficiency, dressed yield, or ready-to-cook yield was observed. A 4.5% increase in gilet yield	4.5%	[70]
<i>A pinnata</i>	India	Sun-dried meal	56 days	5, 7.5, and 10%	5 and 7.5% reduced cumulative BW and average FCR 5, 7.5, and 10% reduced metabolizable CP and Ca. <i>Azolla</i> did not impact digestive organs or carcass characteristics, except for liver weight, which was reduced by 7.5 and 10%.	7.5%	[59]
<i>A pinnata</i>	India	Fresh	28 days	5, 10, 15, and 20%	15 and 10% had the best BW, but it was reduced with 15 and 20%.	10%	[62]
<i>Azolla spp</i>	Malaysia	-	42 days	5, 10, and 15%	15% increased BW and reduced BW gain, while no significant effects were reported for feed intake (FI) and FCR. There was During the starter and finisher phases, 5, 10, and 15% <i>Azolla</i> increased CPD and CFD, but had no significant impact on ash digestibility.	15%	[56]
<i>A pinnata</i>	India	Dried	42 days	0%, 0% + exogenous enzymes, 5%, and 5% + exogenous enzymes	0% + exogenous enzymes, 5%, and 5% + exogenous enzymes had no significant effect on BW but increased FCR and decreased blood ether extract. 5% + exogenous enzymes improved DMD, CPD, and CFD, whereas 0% + exogenous enzymes decreased them. Additionally, 0% + exogenous enzymes reduced phosphorus in bones.	5%	[71]

Species	Country	Processing	Duration	Incorporation rates	Main findings	Rec. levels	References
<i>A pinnata</i>	India	Fresh	42 days	30%	<i>Azolla</i> increased BW by 7.1% and FCR by 53.7% and reduced total production costs by 25.34% and bird mortality by 3%. <i>Azolla</i> improved carcass efficiency percentage and enhanced the taste of chicken. The housefly population around the poultry farm was hardly noticeable, and unpleasant odors were significantly reduced.	30%	[72]
<i>A pinnata</i>	India	Dried	42 days	2.5%, 2.5% with carbohydrase and phytase, 5%, 5% with carbohydrase and phytase	The four diets had no effect on breast meat attributes, live weight, carcass weight, dressed weight, liver weight, or heart weight, but gizzard weight was increased. Generally, the diets increased cooked meat ash, CP, and pH, while reducing cooking yield. <i>Azolla</i> had no significant impact on sensory properties.	5%	[73]
<i>Azolla spp.</i>	Philippines	Sun-dried	35 days	5, 10, 15, and 20%	15 and 20% decreased BW gain, whereas 10% had no significant effect. However, 5, 10, and 15% improved the FCR. <i>Azolla</i> increased feed costs per unit gain in weight and reduced returns above the feed costs.	5%	[58]
<i>A pinnata</i>	India	Sun-dried	42 days	2.5, 5, 7.5, and 10%	2.5, 10, and 7.5% increases in BW gain, ADG, FCR, and PI were observed during the finisher phase. The highest BW gain and ADG were recorded in the 7.5% group. The lowest and best FCR, as well as the highest PI and PER, were observed in the 5% group.	10%	[65]
<i>A pinnata</i>	Nepal	Sun-dried	42 days	5, 10, and 15%	10 and 15% inclusion levels decreased the average cumulative weight and average weekly BW gain, while 15% increased the FCR for all weeks. The economic evaluation of net income per kilogram of live weight was highest in Co, which was on par with 5%. Similarly, the cost of production per bird was lowest in 15% and highest in Co.	10%	[57]

Species	Country	Processing	Duration	Incorporation rates	Main findings	Rec. levels	References
<i>Azolla</i> powder	India	Sun-dried	42 days	5, 10, and 15%	There were significant differences in FCE, FBW, and BW gain. However, there was no significant difference concerning F1 and mortality. 10% Azolla improved dressing percentage, while 15% increased carcass yield.	15%	[55]
<i>A pinnata</i>	India	Fresh	42 days	5 and 10% DM	10% <i>Azolla</i> increased BW at the 5th week and decreased FCR at the 5th and 6th weeks, while no effect was reported on weekly average absolute gains and cumulative weekly feed consumptions. 5 and 10% <i>Azolla</i> showed a higher economic efficiency.	10%	[74]
<i>A pinnata</i>	India	Sun-dried	56 days	5, 7.5, and 10%	No significant effect was reported for the hematological profile, except for lymphocyte and heterophil counts, which were higher in the 5 and 7.5% groups, and eosinophil counts, which were higher in the 7.5% group. Although AST levels were higher in the 10% group, the values remained within the normal range for broiler chickens.	10%	[75]
<i>A pinnata</i>	India	Dried meal	112 days	5% DM and <i>ad libitum</i>	<i>Ad libitum Azolla</i> reduced FCR, while no significant effect was observed on blood parameters except for uric acid, which decreased at a 5% DM inclusion rate. No significant impact was reported on humoral or cell-mediated immune responses.	5% DM	[76]
<i>A. micro-phylla</i>	Indonesia	Fermented	-	10, 15, 20, and 25%	10%, 15%, and 20% had no significant impact on feed consumption, BW gain, FCR, percentage of the carcass, and abdominal fat percentage, except for 25%, which decreased them.	20%	[77]
<i>A. pinnata</i>	India	Dried	42 days	2.5, 5, 7.5, and 10%	2.5, 5, and 7.5% <i>Azolla</i> spp. improved FBW, ADG, BWG, and PI. 2.5 and 5% reduced FCR. 2.5 and 10% increased PER.	7.5%	[103]

Table 3.
 Research concerning the use of *Azolla* spp. in broilers and turkeys.

Species	Country	Processing	Duration	Incorporation rates	Main findings	Rec. levels	References
<i>A. pinnata</i>	India	Fresh	60 days	30-45 g/day	<i>Azolla</i> increased egg-laying capacity (10-15%)	30-45 g/day	[78]
<i>A. microphylla</i> and <i>A. filiculoides</i>	India	Fresh	119 days	10 g/day	<i>Azolla</i> increased egg productivity, total protein, and carotene content, and it decreased the daily intake of concentrated feed.	10 g/day	[21]
<i>A. pinnata</i>	India	Fresh	91 days	200 g/day	<i>Azolla</i> decreased FI and increased Roche fan color score and feed cost, while no significant difference was observed in egg weight or quality.	200 g/day	[79]
<i>A. pinnata</i>	India	Dried	80 days	5% and 10%	No significant difference was reported for duck-day egg production percentage, egg production, FI, FCR, egg weight, net feed efficiency index, performance efficiency index, and cost of feed per dozen eggs [Rs.]. However, there was a saving of about Rs. 3 per dozen egg production in the 10% group. The 10% group also showed an increase in yolk shape and indices, shell thickness, Haugh unit score, and shell percentage.	10%	[80]
<i>A. pinnata</i>	India	Fresh	112 days	200 g per chick per day	No significant effect was reported for mean hen-housed egg production per week and immunocompetence measured in terms of hemagglutination inhibition (HI) titer, Foot Index, and total serum protein concentration. <i>Azolla</i> increased FBW, ADG, and the FCR, and decreased feed consumption by 30.73%, culminating in a 0.76 savings on feed cost per egg per day over the control.	200 g per chick per day	[81]
Rec. recommended							

Table 4.
Research concerning the use of *Azolla* spp. in laying hens, s, and fowl.

The reduced performance at higher inclusion rates (>15%) could be due to decreased feed intake and the presence of antinutritional factors, such as tannins and oxalates. Furthermore, the cost-effectiveness of *Azolla*, as noted in several studies, underscores its value in reducing feed costs while maintaining or improving performance metrics [60–63].

Generally, *Azolla* did not impact carcass characteristics, and regarding meat quality, dried *Azolla* had no significant impact on sensory properties [64, 73]. However, fresh *Azolla* improved meat flavor, juiciness, and tenderness [82]. For laying poultry, *Azolla* either had no impact or improved egg production and quality [21, 78–80, 83], except for one study that reported increased HDL levels [61].

2.2 Weaknesses

Because *Azolla* grows in water, cultivation requires a consistent supply of water, which may not be feasible in arid regions. In addition, there is a risk of overgrowth and the need for maintenance. Without proper management, *Azolla* can overgrow and require meticulous attention to avoid becoming an invasive species. *Azolla* forms dense mats on water surfaces, leading to decreased pH and oxygen levels, increased nutrient concentrations, and reduced macrophyte abundance and richness. This overgrowth can severely affect aquatic ecosystems, impacting various species, including amphibians. For instance, *A. filiculoides* mats have been associated with decreased survival rates for the western spadefoot toad (*Pelobates cultripes*) [84].

Although *Azolla* is high in protein, it may lack the essential nutrients required for a balanced diet, necessitating supplementation with other protein sources. *Azolla* is rich in lysine but deficient in tryptophan and sulfur-containing amino acids [31]. Although *Azolla* contains various minerals, its bioavailability is limited. For instance, despite its high iron content, the iron is primarily present as a less bioavailable ferric cation [85]. Moreover, under stress conditions, *Azolla* species exhibit increased production of phenolic compounds and carbohydrates but decreased concentrations of lipids and total proteins [42]. Additionally, fresh *Azolla* contains a high percentage of a high percentage of moisture (Table 1), necessitating preservation techniques such as drying, which can increase labor and storage requirements. Sun-dried *A. pinnata* demonstrated a dry matter content reaching 93% [29].

The cultivation of *Azolla* and its exploitation as a protein resource worldwide may not be possible due to limitations in management conditions. In general, the optimal relative humidity ranges between 85% and 90%, and the ideal temperature ranges from 20°C to 30°C. This aquatic fern is affected by pH, with the ideal pH range being 5.5 to 7 [86]. For *A. pinnata*, the optimal growth rate of 0.321 day⁻¹, with a doubling time of 2.16 days, was achieved under high light intensity (20,000 lx), a 15% strength of the Hoagland solution, pH control, 90% humidity, and the presence of nitrogen in the medium [87]. *A. filiculoides* exhibited maximum growth at 22°C [27], and when exposed to cold treatment and high light intensity, it developed reddish pigmentation as a protective mechanism [88]. However, these growth conditions stimulated flavonoid accumulation, which likely prevented irreversible photoinhibition-induced damage. The presence of tannins and oxalates in *Azolla* may limit its inclusion at high levels. Processing methods, such as sun-drying or mixing with other feeds, can mitigate these effects.

2.3 Opportunities

As an aquatic macrophyte, *Azolla* offers the widest variety of uses; it could be profitably expanded in the marginal areas of Mediterranean countries as an alternative protein resource for both ruminants and monogastric animals. In addition, its use has ecological and environmental advantages, making it a sustainable option for agricultural practices.

- As an alternative to conventional feed, *Azolla* offers a sustainable option that aligns with the increased interest in eco-friendly agriculture. By reducing reliance on imported or manufactured feed, *Azolla* can contribute to adapting livestock production to climate change by providing locally resilient feed sources.
- *Azolla* cultivation can ensure the creation of new sources of income for small-scale farmers and enhance food security in rural communities.
- *Azolla* is versatile and can thrive in temperate and tropical regions [89]. It grows well in partially treated wastewater [90]. Moreover, some species, such as *A. rubra* and *A. microphylla*, have shown tolerance to saline conditions [91]. *A. pinnata*, an endemic species in India, displays a lower growth rate, biomass production, and nitrogen-fixing ability compared to other species and is unable to maintain growth in salt-containing environments [91].
- *Azolla* is a dual-purpose aquatic fern with significant applications in agriculture and environmental management. It serves as an effective green manure for rice cultivation owing to its ability to fix nitrogen in association with cyanobacteria, providing 30 to 60 kg N ha⁻¹ [92]. At the same time, *Azolla* has shown exceptional potential for bioremediation, particularly for the absorption of heavy metals, including Cd, Cu, Cr, and Zn, from wastewater [89, 92].
- Although the pharmacological applications of *Azolla* remain largely unexplored, it contains tannins, terpenoids, alkaloids, saponins, polyphenols, and flavonoids, all of which possess various pharmacological properties [93]. In an in vitro study, methanolic extracts of *A. filiculoides* and *A. caroliniana* demonstrated antimicrobial activity against certain pathogens, as well as antioxidant, anti-inflammatory, and cytotoxic properties [94]. Moreover, the methanolic extract of *A. microphylla* inhibited the growth of *Xanthomonas oryzae*, which causes bacterial blight in rice.
- Opportunities exist to improve *Azolla* cultivation methods, nutrient content, and storage capabilities through agricultural research and technology.

2.4 Threats

Owing to its rapid growth, adaptability, and high competitiveness, this invasive trait has negatively impacted aquatic vegetation and, generally, water quality [84]. *Azolla* can proliferate quickly and form very thick and dense coverings in favorable environments, leading to many problems for aquatic fauna, hindrance to boat

navigation, and threats to native plant species, in addition to becoming a source of eutrophication. When forming dense mats in temporary Mediterranean waters, *A. filiculoides* can severely alter the chemical and physical properties of the water. It decreases the pH and oxygen concentration while increasing nutrient levels, particularly nitrogen and phosphorus compounds [27]. The presence of *Azolla* mats reduces macrophyte abundance and richness, thereby altering the composition of aquatic plant communities. It also affects the plankton community, increasing phytoplankton abundance and changing zooplankton composition [84]. Therefore, *Azolla* sp. cultivation may face regulatory hurdles and concerns regarding its introduction into new environments.

Azolla has been used as a replacement for traditional technologies in environmental bioremediation [95]. It has been widely used for wastewater decontamination and has resulted in massive biomass production [96]. After completing the phytoremediation process, it is essential to properly handle the resulting biomass, as it may contain harmful elements that could cause serious environmental risks if not managed correctly [97]. In a study comparing pond *Azolla* with cultivated *Azolla*, pond *Azolla* was found to be highly concentrated in Pb, Cd, and Cu [24]. According to **Table 1**, each *Azolla* species was highly concentrated in a heavy mineral (Co, Ni, Cr, Cd, Mo, and Pb), with *A. filiculoides* highly concentrated in Pb (25 ppm). Some authors have suggested the selection of less seed-producing genotypes to control the *invasive potential of Azolla*. However, *Azolla*'s primary mode of reproduction is vegetative through fragmentation [98], and more vigorous genotypes could potentially be more invasive owing to their faster growth rates. Other solutions, such as biological control through the introduction of the weevil *Stenopelmus rufinasus*, have shown positive results, reducing herbicide use and management costs by millions annually [99].

3. Conclusion

As an alternative protein source, *Azolla* has significant potential for improving the sustainability and efficiency of livestock feeding systems. Its high nutrient content, rapid growth, and ability to be cultivated with minimal resources make it a promising candidate for replacing or complementing conventional feed ingredients. The integration of *Azolla* into feeding strategies can enhance livestock productivity, reduce feed costs, and contribute to environmental sustainability by lowering greenhouse gas emissions and mitigating the effects of overgrazing. However, its effective utilization requires addressing challenges such as its invasive nature, high moisture content, and high concentration of antinutritional factors. Processing methods, such as drying, fermentation, or mixing with other feed ingredients, can mitigate these issues and maximize its benefits. The SWOT analysis conducted in this chapter highlights *Azolla*'s strengths and opportunities while emphasizing the need for further research to optimize its production and incorporation into diverse livestock systems. Overall, *Azolla* holds considerable promise for meeting the growing global demand for sustainable livestock production, especially in regions facing feed scarcity and rising costs. Collaborative efforts among researchers, farmers, and policymakers are crucial for unlocking its full potential and ensuring its successful integration into agricultural practices.

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