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Association of Resilience and Production

Edited by Sándor Kukovics



Sheep Farming - Association of Resilience and Production

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Aims and Scope of the Series

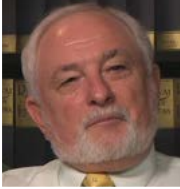
The importance of agriculture cannot be overstated. It helps sustain life, as it gives us the food we need to survive and provides opportunities for economic well-being. Agriculture helps people prosper around the world and combines the creativity, imagination, and skill involved in planting crops and raising animals with modern production methods and new technologies. This series includes two main topics: Agronomy and Horticulture, and Animal Farming. This series will help readers better understand the intricacies of production agriculture and provide the new knowledge that is required to be successful. The success of a farmer in modern agriculture requires knowledge of events happening locally as well as globally that impact input decisions and ultimately determine net profit.

Meet the Series Editor



W. James Grichar has been employed with Texas A&M AgriLife Research for over 45 years with an emphasis on research in agronomy, plant pathology, and weed science. He obtained his BS from Texas A&M in 1972 and his Masters of Plant Protection in 1975. He has published 195 journal articles, over 330 research reports and briefs, 11 book chapters, and over 300 abstracts of profession meetings. He also directs research in many crops including corn, grain sorghum, peanuts, and sesame. He has held various positions in different professional societies including the American Peanut Research and Education Society, Southern Weed Science Society, and Texas Plant Protection Conference in addition to being Associate Editor for Peanut Science and Weed Technology. Significant accomplishments have included spearheading efforts to determine the optimum planting time for soybean production along the upper Texas Gulf Coast. These efforts have shown growers that soybean yields can be improved by 10 to 20% by following a late March to early April plant date. He also has been instrumental in developing a herbicide program for peanut production in the south Texas growing region. Through the development and use of herbicides that are effective against major weed problems in the south Texas region, peanut yields have increased by 25 to 30%.

Meet the Volume Editor



Prof. Dr. Sándor Kukovics spent 40 years in the Research Institute for Animal Breeding and Nutrition (Herceghalom, Hungary), being responsible for the small ruminants sector, edited 45 books, published more than 1,560 articles, and holds licenses for 4 products. He studied at various universities in Hungary. Since 1996, he has been serving as president of the Hungarian Sheep and Goat Dairying Public Utility Association and, since 2010, as executive manager of the Sheep and Goat Products' Board. Between 2015 and 2019, he served as vice president of the EU COPA-COGECA Working Party on Sheep and Goats. Since 2016, he has been a member of the Board of Directors within the International Goat Association. He organized 26 national and 16 international conferences.

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Preface

The resilience of animals is of decisive importance in all life functions. This resilience is influenced by many environmental factors, including the types of feed fed and their nutrient content, the feeding process, animal health, and breeding and environmental factors. Among these, stress plays a significant role, affecting all physiological functions, including resilience and production efficiency, as well as the quality of semen, which is of decisive importance in reproduction.

Environment and Stress

Animals are exposed to many stresses throughout their lives, which influence their behavior, production, well-being, and resilience.

Stress factors directly and/or indirectly affect the biological functions of the animal, impairing the immune system, performance, productivity, product quality, and increasing mortality rates. Stress factors and the costs they impose increase the cost of animal husbandry and are therefore considered economically important in sheep farming.

Early identification of stressors and effective stress management, along with the development of appropriate strategies to improve animal welfare and achieve optimal production, are of utmost importance in animal husbandry. Chapter 1 provides comprehensive information on stress factors, early recognition of stress and its impact on performance, the behavioral and physiological changes it causes, and the potential for using this knowledge.

Nutrition

The digestive properties of ruminants and the feed they consume greatly influence their greenhouse gas emissions. They try to modify and fundamentally reduce methane gas emissions by applying several methods. In the second chapter, the effects of biochar application on reducing methane emissions and on growth and weight gain were investigated. Not only did they reduce methane emissions, but they also increased feed utilization efficiency, according to the study.

Sheep feed is based on various plant species. In dry, water-deficient areas, succulent plants can play a particularly important role, as their phytochemicals and water content greatly influence the nutritional supply and the resistance of the fed animals. The applicability and effects of these plants in sheep feeding are presented in Chapter 3. Their use as feed supplements not only increases the efficiency of animal feeding but also improves animal resistance and health in the given production and husbandry environment and even reduces methane emissions from animal husbandry.

Some aquatic plants can also play an important role in sheep feeding, if they are properly muddled, treated, and added to the animal feed in the desired condition. In the fourth chapter, the collection, drying, treatment, and use options for *Azzola Pinnata*, as well as its effects on animals, were analyzed. According to the results, the tested plant improves digestion efficiency in both its dried and original forms, thereby positively affecting the animals' resistance.

Reproduction

Determining the element of the reproduction and breeding of sheep, as well as the genetic development, is the quality and quantity of semen produced by male animals, which is not only directly related to the animals' resilience, but also determines their successful reproduction.

This is especially important in the case of animal artificial insemination. The quantity and quality of semen are influenced by many factors, like breed, age, feeding and husbandry technology, the animal's health condition, the season of semen production, possible heat stress, the method of semen collection and treatment, as well as preservation and storage, and the methods used in artificial insemination.

The factors affecting semen quality have been summarized and analyzed in Chapter 5.

Animal Health

In addition to meat, milk, and wool, the usability of the hide of slaughtered animals is also of great importance in the production of sheep products. Together, these products determine the economic efficiency of sheep farming and product production.

The quality and usability of the hide are influenced by several factors, including parasites, bacteria, fungi, viruses, and mineral deficiencies, which have been implicated in causing cutaneous lesions in sheep farming. The treatment of the consequences of these not only incurs additional costs but also worsens the hide's usability.

In addition, skin and tissue diseases compromise animal health and resilience.

In the sixth chapter, the diseases affecting the quality of sheep hides, their effects, and possible treatments were summarized.

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Section 1

Environment and Stress

Chapter 1

An Early Warning Indicator for Stressors in Sheep and Their Impacts on Productivity: An Updated Review of Behavioral and Physiological Responses to Stress Factors

*Emmanuel Malematja, Sekobane Daniel Kolobe,
Monnye Mabelebele and Jones Wilfred Ng'ambi*

Abstract

Sheep play a significant role in socio-economic development across the world by ensuring food security and poverty eradication. Sheep are versatile and their adaptability to various climatic conditions is demonstrated by their presence in different geographical regions. However, due to ever-changing environmental conditions and today's livestock practices, sheep are exposed to distress while trying to keep pace with climate change. Distress is defined as a situation in which the animal shows an overt behavioral sign of suffering. Stress factors such as heat, cold, and on-farm stress factors are stimulants that initiate a stress response in the animal and are unavoidable due to our modern livestock practices. The physiological and production status, breed, age, sex, stress factor, and its duration determine the animal's response to a particular stressor. Stress factors directly and/or indirectly affect the biological functions of the animal, subsequently compromising the immune system, performance, productivity, product quality, and increasing mortality. Stress factors constitute the cost of stress, and they are, therefore, considered to be economically important in sheep farming and the red meat industry. Hence, it is important to identify and address stress factors at an early stage to manage distress effectively, as well as to develop adequate strategies to improve animal welfare and achieve optimal production. Therefore, this chapter seeks to provide comprehensive information on stress factors, early detection of stress, and the impact of stress on performance. Moreover, the chapter discusses the potential of using observations of behavioral and physiological responses to recognize stress factors.

Keywords: animal welfare, distress, stress factors, productivity, stress indicators

1. Introduction

Small ruminants play an important role in food security, socio-economic development, and poverty eradication for rural and peri-urban smallholder farmers in both developing and underdeveloped countries [1]. About 70% of the small ruminant population thrives well in arid and semiarid regions [2]. Sheep are among the highly adaptable ruminants, and due to their versatile adaptability characteristics, sheep are spread throughout various geographical regions worldwide [3], where they are raised for several reasons, including meat, milk, leather, income, and wool [4]. However, the expansion of the ruminant livestock sector, along with climate change scenarios, has exposed sheep to a variety of stressors such as heat, cold, and on-farm stress factors, including animal handling, nutrition, transport, shearing, and weaning, that they must cope with to maintain optimal productivity [5].

To ensure productivity, animal welfare must be considered, as inadequate conditions can negatively affect the performance of the animal [6]. Extreme temperatures and on-farm managerial aspects pose a significant challenge to sheep and can lead to physiological and behavioral changes that compromise growth performance, reproduction, and the ultimate product [7]. Climate change remains the major threat to the thriving sheep industry and its sustainability, particularly in tropical and temperate regions [8]. The ability of sheep to do well depends on the animal's capacity to cope with the changing environment [4]. The failure to adapt to harsh environmental conditions triggers stress, which results in negative effects on physiological functioning, reproduction, the immune system, and growth performance [7]. Moreover, sheep will go through various stress levels, either psychological and/or physiological, throughout their lives in production [5, 9]. Stress is defined as a reflex reaction that occurs when the animal's physiological function fails to cope with the changing environment [10]. The effects of stress can range from discomfort to death [8].

Additionally, stressors have negative effects on a variety of productive parameters such as growth performance, reproduction, wool, carcass traits, and milk yield [11]. Adverse effects of stress factors, such as sickness in animals, can lead to the use of drugs in food-producing animals [9]. Under climate change scenarios, multiple stress factors emerge as a potential threat to sheep productivity and their survival [12], and this necessitates the need to study and understand these stress factors to mitigate them simultaneously to improve the economy of sheep farming [2]. Thus, this chapter discusses the potential impact of stressors and the possible use of observation on behavioral and physiological responses to recognize stress factors and/or distressed animals and provide strategies to deal with stress factors in sheep.

2. Methodology

To achieve the objectives of this chapter, the authors performed a literature search on electronic databases such as Google Scholar, Science Direct, JURN, ResearchGate, and the Directory of Open Access Journals, using the keywords "adaptability," "behavioural response," "physiological response," "sheep," and "red meat" to review observational studies conducted in sheep on procedures that induce

behavioral and physiological stress responses. Identified articles were subsequently evaluated and sorted according to relevance. In addition, an analysis was conducted using the keywords employed in the literature search to examine the association between the stressors and their effects on the productivity of the animal using VOS Viewer [13].

3. Results and discussion

3.1 Different types of stress and their impact on animals' productivity

Various stressors and their adverse effects on sheep animal welfare, reproduction, performance, and product quality have been reported in the literature. A stressful state in farm animals can be described as a unique event that evokes a specific behavioral, physiological, neuroendocrine, and/or immune response to a specific stressor, which may be as unique as the stressful state [10]. **Figure 1** represents an analysis of the association between the stressors and their effects on the productivity of the animal. There is a strong association between stress, animal welfare, and performance.

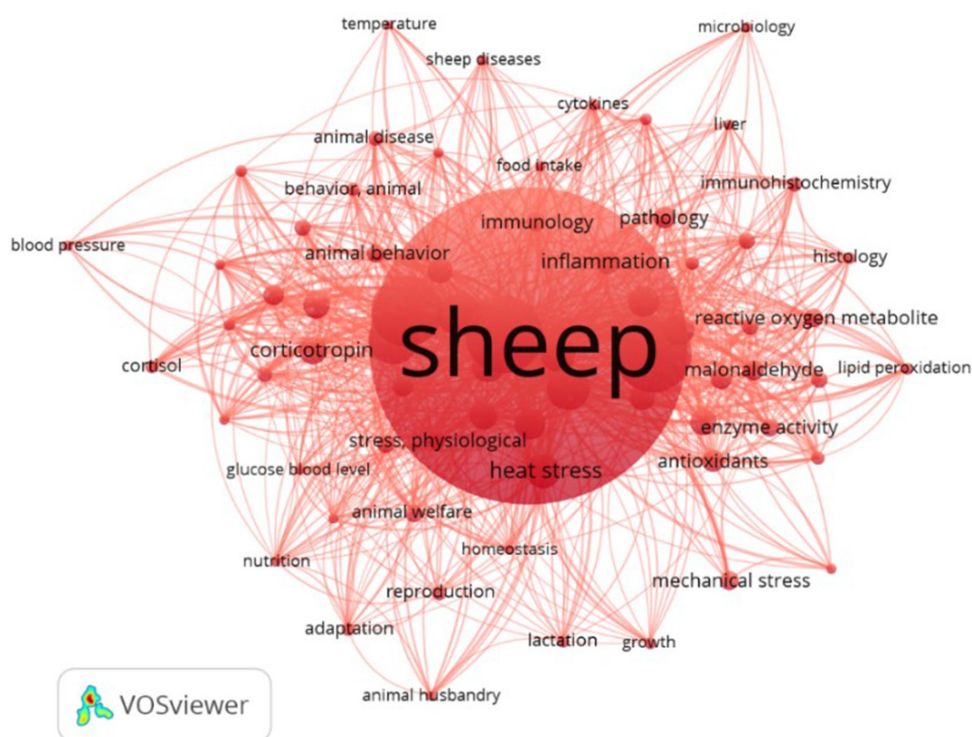


Figure 1.
 A network map representing the bibliometric analysis of stressors in sheep farming and their contribution to animals' behavior, health and welfare, production, and reproduction. This figure was generated on VOS viewer [13], using studies conducted on the effects of stressors in sheep.

3.2 Ambient temperatures

Each animal species has its own temperature range for a comfort zone, ideal for optimum productivity and temperature sensitivity, and this might vary within the same species depending on age and physiological state [4]. The thermal comfort zone in sheep ranges between 15 to 30°C [14], and beyond 35°C is considered the critical temperature [15]. Although sheep are versatile and highly adaptable to wintry conditions compared to other farm animals, their ideal temperature should not be below -20°C [16]. Additionally, the ideal conditions may vary widely between sheep breeds, shorn and unshorn sheep, and in wind and/or rain [16]. Cold stress is often ignored in livestock as the focus is mainly on heat stress and its negative effects [7].

In **Figure 2**, the ambient temperature ranges are split into different zones with respect to the thermal comfort of the sheep. In the thermoneutral zone (15°C to 30°C), the sheep will not experience lower critical temperature nor upper critical temperature, and their health, welfare, and productivity are optimal [2]. However, as the ambient temperature changes, for instance, by rising (30°C–35°C, point C), the sheep will start feeling warm (warm zone) and subsequently reach the upper critical temperature zone (beyond 35°C, point D), which is the temperature above which thermoregulatory processes activate evaporative heat loss mechanisms in sheep [7, 17]. On the contrary, as the ambient temperature drops below the comfort zone (15°C–10°C), the sheep will start to feel cool (cool zone, depicted by point B) and eventually enter a state of cold stress when the ambient temperature drops below 10°C (lower critical temperature indicated by point A), where the sheep rely solely on thermogenesis to maintain their body temperature. When the body temperature exceeds or drops below the thermal zone (15°C–30°C), the physiological functions of the sheep are affected, causing adverse effects on the productivity of the animal [2, 7]. Therefore, ambient temperatures represented by points A and B indicate the limits for the occurrence of ambient temperature stress.

Heat stress occurs as a result of imbalances between metabolic heat generation (internal) and its dissipation to the surroundings (external) under extreme hot and

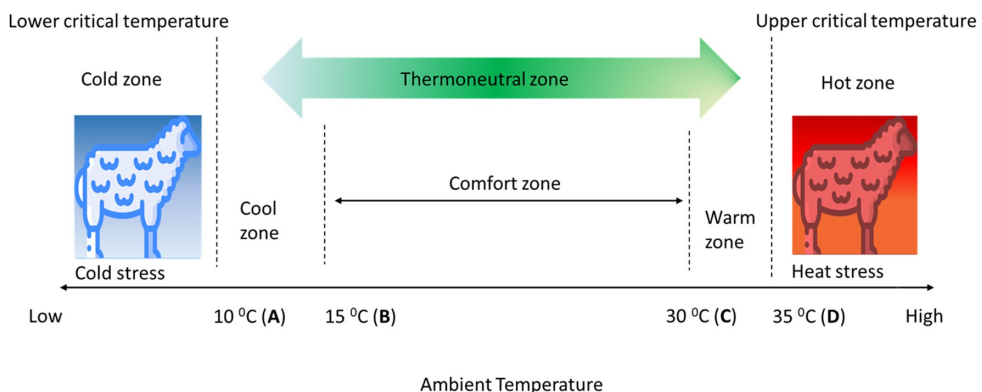


Figure 2. Schematic representation of ambient temperature stress and response mechanisms to changes in ambient temperature (developed by authors).

humid conditions [8]. Although goats and sheep are versatile and can easily adapt to heat stress better than most ruminants, their productivity is often compromised [18]. In arid and semiarid regions, summer seasons are warm ($\geq 40^{\circ}\text{C}$) and dry with little or no rainfall, while autumn and spring are thermoneutral, and winter is slightly cold [19]. Hence, heat stress prevails in summer and remains a major key factor for lower productivity in sheep farming based in arid and semi-arid ecological regions [19]. Higher ambient temperatures (beyond 35°C) have adverse effects on animal performance, reproduction, and welfare [11, 20]. This is supported by Nicolás-López et al. [20], who observed a significant reduction in blood serum from Dorper x Katahdin sheep subjected to chronic heat stress. Higher ambient temperatures have lowered dry matter intake in sheep [15]. Furthermore, it is evident that heat stress is a limiting factor for breeding in modern sheep breeds [11]. However, heat stress did not affect blood urea nitrogen and glucose concentration in fattening lambs [11].

Exposure to lower temperatures, especially during the winter season, led to cold stress and subsequently resulted in hypothermia in the animal [21]. Cold stress is one of the biggest stress factors in winter, as low temperatures increase energy expenditure in animals, particularly in ruminants [22]. Exposure to cold stress has been reported to deteriorate growth performance, production, and reproduction in ruminants [7, 11]. Similarly, Kang et al. [23] evaluated the effect of ambient temperature on ruminants and reported that colder ambient temperatures have adverse effects on growth and feed efficiency. On the other hand, there was no evidence of adverse effects on mature European sheep breeds exposed to low temperatures below -20°C with higher precipitation and relative humidity [16]. When animals are experiencing cold stress, increasing feed intake helps to compensate for the body heat through metabolic processes [24]. Studies conducted on the impacts of ambient temperature on growth, hematology, and metabolism in sheep are summarized in **Table 1**.

Breed	Stress factor	Parameter	Effect	Author
Malpura	Heat	Growth	Did not affect ADG in rams; however, DMI was increased	[25]
Malpura crossbred	Heat	Feed intake/ grazing	Heat stress had reduced DMI and ruminating while animals were lying down. However, the animals spent more time ruminating while standing	[15]
Dorper x Katahdin	Heat	Growth	Reduced growth rate and feed efficiency, with no effect on DMI	[20]
Dorper x Katahdin	Heat	Physiological response	Increased rectal temperature and respiratory rate compared to thermoneutral	[20]
Dorper x Katahdin	Heat	Hematology	Lowered erythrocyte and platelet counts, hemoglobin, and hematocrit. However, heat stress increased erythrocyte size and leucocyte count compared to the thermoneutral group	[20]
Dorper x Katahdin	Heat	Blood serum	Heat stress reduced glucose, cholesterol, total protein, urea, and potassium concentrations compared to thermoneutral conditions	[20]

Breed	Stress factor	Parameter	Effect	Author
Afshari sheep	Heat	Growth	Reduced ADG with no effect on DMI in fattening lambs under moderate heat stress; however, severe heat stress led to adverse effects on DMI and ADG	[11]
Afshari sheep	Heat	Physiological response	Heat stress elevates body temperature and respiratory rate	[11]
Afshari sheep	Heat	Metabolism variables	Did not influence plasma glucose or blood urea nitrogen	[11]
Dorper × Mongolian	Cold	Growth performance	Exposure to cold stress conditions resulted in reduced ADG and an increase in DMI, as well as the F:G ratio, compared to thermoneutral	[26]
Dorper × Mongolian	Cold	Hematology	Cold stress conditions had significantly reduced immunoglobulin and interleukin levels	[26]
Small-tailed Han sheep × Hu sheep	Cold	Growth	Exposure to chronic cold stress reduced ADG in sheep	[27]
Small-tailed Han sheep × Hu sheep	Cold	Hematology	Low temperatures caused a significant reduction in immunoglobulin compared to thermoneutral conditions	[27]
Mongolian sheep ewes	Cold	Growth performance	Subjecting animals to the cold season resulted in lower body weight than that in the warm season	[28]
Mongolian sheep ewes	Cold	Physiological response	The rectal temperature was not affected by the changes in seasons	[28]

ADG: average daily gain; DMI: dry matter intake; F:G ratio: feed-to-gain ratio.

Table 1.
Summary of the effects of ambient temperature on production parameters in various sheep breeds.

3.3 Plane of nutrition

Global warming has direct adverse effects on the livestock sector in terms of feed and water availability and quality, disease outbreaks, and increased animal husbandry challenges [12, 18]. Clean and freshwater are essential for the maintenance of the body, as they play a significant role in physiological functions for optimal productivity in farm animals [6]. Furthermore, the scarcity of feed presents a major factor for lower productivity of sheep in arid and semi-arid areas [29].

Seasonal changes in nutrition remain the biggest challenge in sheep farming. Optimal nutrition increases the adaptability of the animal to adverse environmental conditions by ensuring an adequate supply of essential nutrients [18]. In contrast, insufficient pastures have adverse effects on the biological functioning of the animal [1, 4]. Maurya et al. [12] carried out a study on feed restriction in rams and reported lower dry matter intake and higher water intake. However, the authors reported no alteration in average daily gain in response to nutrition restriction compared to the control group. A delay in feeding did not alter body conditioning and physiological responses of lambs [30]. Summaries of studies investigating the impacts of nutrition on sheep breeds are presented in **Table 2**.

Breed	Stress factor	Parameter	Effect	Author
Not specified	Water intake	Growth performance	Increased water intake led to a significant reduction in DMI	[6]
Malpura	Nutrition and thermal	Growth performance	Increased water intake in ewes	[29]
Malpura	Nutrition and thermal	Respiratory system	Plane of nutrition did not affect respiration rate in the early hours; however, respiration rate was reduced in the afternoon	[29]
Malpura	Nutrition and thermal	Pulse rate	Did not affect the pulse rate in ewes	[29]
Malpura	Nutrition and thermal	Rectal temperature	Reduced rectal temperature in the morning and afternoon	[29]
Malpura	Nutrition restriction	Growth performance	Reduced DMI and water intake in rams	[25]
Malpura	Nutrition restriction	Growth performance	Did not affect ADG	[25]
Chios and Karagouniko lambs	Feed provision times	Growth performance	Delay in feeding did not affect the body condition of the lambs	[30]
Tibetan Plateau	Feed deficiency	Growth performance	Lambs on natural grazing had lower DMI, ADG, and FBW when compared to those who received supplementation or barn feeding	[31]
Jamuna Basin lambs	Nutrient deficiency	Carcass traits	Hot carcass and dressing weight were increased by concentrate supplementation compared to the control group	[32]
Jamuna Basin lambs	Nutrition deficiency	Growth performance	Incremental levels of concentrate supplementation increased ADG and slaughter weight in lambs compared to the group that received no supplementation	[32]

ADG: average daily gain; DMI: dry matter intake; FBW: final body weight.

Table 2.
Effects of nutrition on production parameters in various sheep breeds.

3.4 Transport stress

Transporting live animals from point A to B safely, humanely, and efficiently ties together animal welfare, profitability, and public trust. However, transport induces stress in farm animals. Transport stress occurs due to transport durations (long journeys), and this stress factor is unavoidable and plays a significant role in sheep farming [33]. During transport, the most stressful period is at the start of the trip, and long journeys may cause fatigue [11, 34]. It has been reported that transporting sheep for one hour caused less stress compared to two hours of transportation [35]. The conditions provided prior to slaughtering, including loading, transporting, unloading, and treatment of the sheep, contribute to stress [4]. During the transportation of animals from the farm or market to the abattoir, poor road conditions and transport vibrations can lead to a stressful situation [34].

Transportation of live animals has a significant impact on welfare and may cause economic loss due to the adverse impact on welfare and carcass quality [33]. During transport, animals are not provided with water and feed, and as the journey duration increases, the degree of nutritional stress increases, as do thirst and environmental stress [33]. Fasting sheep prior to transport intensified stress during the winter season [35].

It has been reported that sheep lose weight during transportation, which leads to economic losses [7]. The physiological stress due to the transportation of sheep is reflected in blood constituents such as cortisol, glucose, plasma urea, and creatine kinase [7]. Further, the physical impact of transportation includes bruising, while other effects of transportation on sheep are dehydration and depletion of glycogen [7]. It has been documented that both physiological and physical responses to stress have adverse effects on meat quality attributes [7]. However, it should be noted that the severity of these effects depends on the age of the animal and the transport facility [33]. Further, older sheep lose more weight during transportation than younger ones [7]. Ewes lost approximately 1 kg of live weight and had higher body temperatures after a 12-hour journey when compared to young animals [36].

Transporting animals with or without lairage did not affect pre-slaughter weight in Syrian Awassi lambs [37]. Zhong et al. [7] investigated the effects of transportation in the sheep industry on blood parameters and observed no significant differences in total protein, albumin, globulin, and total cholesterol in sheep transported for about 8 hours. Similarly, transportation duration (1 or 24 h) did not affect blood parameters, with the exception of blood urea nitrogen, which was higher in animals transported for one hour [34]. Contrary to these findings, Pascual-Alonso et al. [36] demonstrated that even short journeys could induce behavioral, physiological, and thermo-physiological responses that can lead to weight shrinkage in ewes. Studies investigating the effects of transportation stress on growth performance, health, welfare, and meat quality in lambs are summarized in **Table 3**.

Breed	Stress factor	Parameter	Effect	Author
Ujumqin sheep	Eight-hour journey	Growth parameters	Reduced body weight and hemoglobin; however, serum urea concentration was increased	[7]
Ujumqin sheep	Eight-hour journey	Carcass weight	Increased carcass weight and dressing percentage in the transportation treatment compared to the control group	[7]
Ujumqin sheep	Eight-hour journey	Meat attributes	Transportation did not affect meat pH or meat shear force. However, glucose was reduced when compared to the control	[7]
Red Karaman, İmroz, Sakız, and Karakul rams	Seventy-five-minute journey	Behavior	During the 75-minute transport, Red Karaman rams exhibited higher vocalization compared to Imroz, Sakız, and Karakul rams	[38]
Merino lambs	Twenty-hour journey	Carcass traits	Transporting lambs for 24 hours did not affect the cold-carcass weight compared to those transported for 1 hour	[34]
Kıvrıkcık lambs	Transport duration	Behavior	Transporting lambs for 45 and 120 minutes did not alter the behavior behavior in terms of walking, lying down, and vocalization	[39]

Breed	Stress factor	Parameter	Effect	Author
Chamarita adult	Transport	Physiological response	Increased internal body temperature in ewes compared to the control group	[36]
Dorper × Mongolian	Two-hour journey	Blood parameters	Long transportation did not affect blood creatine kinase in sheep	[35]
Syrian Awassi	Transport facility	Growth parameters	Lambs transported without or with lairage did not affect the slaughter weight	[37]
Syrian Awassi	Transport facility	Carcass parameters	Transporting lambs without lairage has reduced carcass shrinkage compared to those transported with lairage. However, hot and cold carcass weights and dressing percentages were not affected	[37]
Dorper × Mongolian	One- and two-hour journey	Rectal response	Rectal temperature was not affected by transportation	[33]
Dorper × Mongolian	One- and three-hour journey	Blood parameters	Total protein, creatine kinase, cortisol, urea, cholesterol, and albumin concentrations were not affected	[33]

Table 3.
Effect of transportation on animal welfare and carcass parameters in sheep breeds.

3.5 Weaning stress

In sheep farming, lambs are weaned when they are 40–50 days old [30]. Weaning is an important managerial aspect in animal husbandry, which influences the later growth rate of lambs [40]. Early weaning can be defined as a widely accepted practice of withdrawing young animals from suckling milk before the usual time to enhance productivity [41], but this practice is often associated with undesirable stresses and reduced performance in lambs [40]. During the weaning process, lambs may experience difficulties in adapting to changes in diets and environments, as well as experiencing weaning shock [30]. Weaning animals not only creates a highly stressful situation for both ewes and lambs but also presents a potential effect on the overall health of lambs [41]. The survival of newborn lambs relies on the establishment of a strong bond and lasting social connection with the ewes [41]. Furthermore, weaning lambs creates a complex stress as it involves dietary changes, environmental changes, and social changes [10], and lambs must adapt rapidly to cope with these changes to remain productive [30]. The dam–lamb relationship is characterized by a long-lasting strong bond and mutual emotional attachment [42]. Therefore, abrupt weaning or separating the dam and lambs provokes stress responses that cause adverse effects on feed intake, weight gain, locomotion behaviors and resting patterns [43]. Additionally, abrupt weaning poses a threat to immunological status and exposes both the dam and lambs to diseases such as mastitis and parasitosis [43]. Weaned lambs increase vocalization, rumination, and water consumption when compared to non-weaned lambs [40]. In contrast, Ekiz et al. [39] investigated the effect of weaning status on changes in behavior and reported no changes in behavior behavior of weaned lambs compared to non-weaned lambs. However, the level of stress hinges on multiple factors such as age, sex, and

Breed	Stress factor	Parameter	Effect	Author
Kivircik lambs	Weaning	Blood parameters	Both weaned and non-weaned lambs had similar plasma concentrations	[39]
Patanwadi lambs	Weaning	Behavior	Weaned lambs increased vocalizations and playing time compared to non-weaned lambs	[40]
Patanwadi lambs	Weaning	Growth performance	Weaned lambs had higher DMI and water intake than non-weaned lambs	[40]
Chios and Karagouniko lambs	Post-weaning	Behavior	Weaning lambs weaned in the evening had decreased vocalization	[30]
Chios and Karagouniko lambs	Post-weaning	Respiratory system	Did not affect the heart rate	[30]
Chios and Karagouniko lambs	Post-weaning	Growth performance	Weaning age did not affect the body weight of lambs	[30]
Neonatal male Hu lambs	Post-weaning	Hematological responses	Early weaning increased white blood cells, lymphocytes, and neutrophils in lambs compared to late weaning	[44]
Neonatal male Hu lambs	Weaning	Immune indices	Early and post-weaning reduced immunoglobulin levels when compared to non-weaned lambs	[44]
Neonatal male Hu lambs	Early and post-weaning	Antioxidant	Early weaning and post-weaning did not alter superoxide dismutase, glutathione peroxidase, or malondialdehyde compared to non-weaned lambs	[44]

DMI: dry matter intake.

Table 4.
Effect of weaning procedures on growth performance, health, and welfare in lambs.

genetic status of the animal. Additionally, the weaning age of lambs varies according to age, weight, and type of food used for weaning [43]. Therefore, understanding the stress factors brought by weaning, the response to these stresses, and how they affect weaning outcomes is essential for sheep farming [43]. The effect of weaning procedures on growth performance, health, and welfare of lambs is presented in **Table 4**.

3.6 An early warning indicator for stress factors in sheep

Behavioral changes and physiological responses to various stress factors have been used as early indicators of stressful situations [10]. Through careful examination of behavioral changes and physiological responses, farmers are able to identify the stress factors and eliminate those that contribute to stressful situations in livestock [9]. However, the behavioral changes and physiological responses to a particular stress factor are highly dependent on the physiological and production status, breed, age, sex, the stress factor itself, and its duration [38]. The impact of stress is clearly reflected by functional abnormalities and changes in behavioral that alter physiological responses brought about by the stress factor [5].

3.6.1 Behavioral changes in response to stressors

As an indicator of distress, changes in the expression of stereotypic behavior, vocalization, and motor activity are early indicators of distress in animals [10]. Furthermore, changes in an animal's behavior could be an indicator of the earliest adaptation and response to changes in environmental conditions [15]. Some of the commonly seen behavioral alterations to counteract the adverse effects of heat stress in ruminants include reducing dry matter intake, rumination time and frequency, and increasing water intake in order to maintain thermal equilibrium [18]. The authors further stated that these behavioral alterations aid in reducing metabolic heat production.

Exposure to higher ambient temperatures (in summer) initiates an intense thermoregulation response to dissipate extra heat [18]. Observable behavioral changes include ruminating, walking, and standing. In the study by Li et al. [45], it was found that ewes would rather ruminate while walking during extreme temperatures, whereas rams tended to ruminate while standing. In addition, ewes and lambs separated from each other during the weaning process become frustrated and stressed, expressing their distress by bleating and increasing locomotor activities [10]. It has been reported that lambs display behaviors to seek and reunite again during the first days after ewe-lamb separation [42]. Therefore, understanding these behavioral changes will aid in recognize distress in sheep. Common observable behavioral changes are summarized in **Table 5**.

3.6.2 Physiological response to stressors

Stress factors are perceived as a potential threat to the animal's body balance [9], and warm-blooded animals have mutated and evolved a variety of mechanisms for adaptation to wider ranges of ambient temperatures [27]. However, if the stressor persists and disturbs the animal's homeostasis, it could result in oxidative stress [5]. When animals are challenged with stress, they develop varying degrees of stress responses. In response to a stressful situation, the animal's physiological response begins in the brain with a warning by the central nervous system through a variety of hormones as well as various neurotransmitters and neuropeptides [5]. In the brain, the hypothalamic-pituitary-adrenal axis, together with the autonomic nervous system and sympathetic-adrenal-medullary axis, regulates the stress response by monitoring the physiological functions according to the course and specificity of the stress that triggers the reaction [5]. In a stressful situation, various endocrinal changes and responses take place to overcome the stressful situation [10]. Physiological responses are means of coping with stressors, and these responses include changes in respiratory rate, sweating rate, pulse rate, and rectal temperature [46]. In response to elevated environmental temperatures, the animals increase their respiratory rate, heart rate, and rectal temperature [8, 18]. Nicolás-López et al. [20] investigated the effects of heat stress on Dorper x Katahdin male lambs and reported an increase in internal body temperature and respiratory rate in response to heat stress compared to the thermoneutral state. However, the basal respiratory rate and degree of responses to changes in ambient temperature are largely influenced by the sheep breed, whereby temperate breeds tend to have a higher basal respiratory rate than Middle Eastern breeds [6]. Hair breeds subjected to chronic heat stress conditions of

Stressor	Behaviour	Meaning	Indicator	Author
Heat	Lying down	By lying down, one helps to reduce the heat load by providing a ready conduit for heat transfer to the floor or ground	Thermo-physiological	[45]
Heat	Reduced DMI	Avoiding metabolic heat production	Thermo-physiological	[18]
Heat	Increased water intake	Animals tend to increase water intake when failing to maintain their thermal equilibrium	Thermo-physiological	[18]
Heat	Reduced rumination time	Avoiding metabolic heat production	Thermo-physiological	[18]
Weaning	Increased locomotion behavior	Display behaviors for reunite	Dam–lamb separation	[18]
Cold stress	Shivering	Rapid twitching of muscle groups anywhere on the body generates body heat	Cold Zone	[17]
Psychological	Grinding teeth/immobility	While other animals are intolerant of pain and/or painful procedures and vocalize, sheep grind their teeth and remain silent rather than vocalizing.	Pain	[17]
Post-weaning	Decreased vocalization	Animals are emotionally stressed and seek to reunite	Dam–lamb separation	[30]

Table 5.

Summary of stressors, common observable behavioral changes, and their possible meaning as an early warning indicator of stress in sheep.

summer reduce their growth rate as well as adjust the physiological, metabolic, and endocrine processes to avoid a hyperthermic state [20]. It has been reported that transportation increases internal body temperature in ewes [36].

An analysis of the association between stressors and physiological responses in sheep was conducted using electronic databases to examine the linkages between stressors, physiological responses, and their effects on the animals' welfare and productivity. In **Figure 3**, the largest circles represent the most frequently used terms by authors, including oxidative stress, physiology, physiological stress, lamb, blood, heat stress, and weaning. Through this bibliometric mapping, we were able to classify these terms into three distinct sets. The blue group represents stressors and their effects on biological functioning in animals. The green group signifies the interaction between physiological response mechanisms to specific stressors (heat, weaning, etc.) and their effects on blood, animal welfare, reproduction, and ewe and lamb performance. The red group demonstrates the association between stressors, physiological responses, and oxidative stress, as well as its effects on immune response. There are strong associations between stressors (heat, weaning, etc.), physiological responses, and growth, health, and welfare indicators. Furthermore, oxidative stress is highly associated with antioxidants, immune system response, apoptosis, malondialdehyde, and glutathione peroxidase.

Conflict of Interest

No potential conflict of interest was reported by the authors.

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Section 2

Nutrition

Chapter 2

Biochar-Enhanced Diets in Naeemi Sheep: A Strategy for Enteric Methane Mitigation and Growth Optimization

Hana'a A. Burezq, Fatin Khalil, Waleed Al-Qalaf and Omar Al-Saeed

Abstract

This study aims to evaluate biochar's potential to reduce methane emissions that contribute to global warming and to examine its effects on enteric fermentation. Recent estimates show that agricultural methane emissions in the EU-15 are roughly 10.2 million tons annually, making it the largest source of such emissions. About two-thirds of these originate from enteric fermentation, while one-third comes from livestock manure. In this research, methane emissions from ruminants were measured in two groups of Naeemi ewes. The treatment group received a diet with added biochar alongside a standard diet, while the control group was given only a standard diet. On day 0, methane emissions of 1,070 ppm were recorded. The addition of biochar to the diet significantly reduced CH₄ emissions by 70.79–74.24% ($P < 0.05$). Additionally, animals in the treated group showed statistically significant improvements in body weight ($P < 0.05$), measuring 64.19 kg, along with notable increases in body length, heart girth, and height at the withers, which were 80.49 cm, 82.95 cm, and 100.49 cm, respectively, compared to the control groups. Biochar appears to have the potential to enhance nutrient utilization, improve feed efficiency, and reduce methane emissions in animals. As a feed supplement, biochar may positively influence digestion and improve the ruminant's capacity for nutrient absorption.

Keywords: sheep, methanogenesis, global warming, biochar, greenhouse gases

1. Introduction

Global food security and sustainable agriculture are urgent issues amid rising demand and climate change. While expanding crop and livestock production is vital for meeting nutritional needs [1], the environmental impacts, especially from the livestock sector, must be addressed. Livestock accounts for approximately 14.5% of total human-caused greenhouse gas (GHG) emissions (~7.0 – 7.1 Gt CO₂ equivalent/year), with methane (CH₄) and nitrous oxide (N₂O) being the main pollutants [2].

Methane from enteric fermentation alone constitutes nearly 39–40% of livestock GHG emissions, with the remainder originating from manure, feed production, and land-use practices [3, 4]. Since methane has 28–36 times higher global warming potential than CO₂ over 100 years, reducing emissions in livestock systems is crucial [5].

Several dietary strategies are under study, including the use of biochar, a carbon-rich material produced by pyrolyzing organic waste at 250–600°C in low-oxygen environments [6]. Meta-analyses and recent trials indicate that biochar can improve nutrient utilization, feed efficiency, and gut health, while potentially reducing methane emissions in ruminants [7, 8].

A 2024 study on specific biochars for cattle confirmed positive effects on rumen fermentation and microbial populations, although the impact on CH₄ emissions varied depending on the type and dose of biochar [9]. Similarly, a 2022 study published by Jindo et al. found that reducing methane emissions through the addition of biochar in compost made from poultry and cattle decreased emissions by 3.0 times for poultry compost but by 3.7 to 4.6 times for cattle compost [10]. A broader review in *Animal Feed Additives* (2023) reported improvements in digestion, weight gain, and methane reduction across various livestock species.

Mechanistic studies suggest that biochar's high surface area and cation-exchange capacity facilitate the adsorption of hydrogen ions (H⁺) produced during enteric fermentation. The shift in microbial metabolism from methane generation to ammonium production results in ammonium adsorption onto biochar surfaces, thereby decreasing CH₄ emissions [11, 12]. The effectiveness of biochar depends significantly on its properties, including particle size, porosity, and surface chemistry [2, 8].

This study investigates the effects of sterile date-palm biochar added to sheep diets, hypothesizing that it will suppress enteric methane emissions by diverting fermentation processes toward ammonium production; enhance rumen fermentation efficiency, nutrient utilization, and animal health; and improve fecal consistency and manure quality. By achieving environmental and productivity goals, this research contributes to climate-smart livestock production and sustainable diets.

We propose that incorporating biochar into livestock feed could potentially reduce methane emissions while also promoting animal welfare.

2. Materials and Methods

2.1 Animals

The field study was conducted at the Kuwait Institute for Scientific Research (KISR) facilities. A total of 28 adult ewes were randomly divided into two equal groups, with 14 ewes per group. Each ewe was housed in an individual enclosure and received two equal portions of feed per day, specifically at designated times in the morning and evening, totaling 0.8 kg of feed per day for each individual. There were no significant differences between the two groups in any respect; however, one group was fed a standard diet alone, while the other group was fed a standard diet supplemented with biochar.

2.2 Preparation of standard diet

Preparation of the standard diets, which consist of 70% concentrates and 30% roughage, is essential.

2.3 Production of biochar

Date palm fronds were obtained from the Kuwait Institute for Scientific Research and then reduced to the appropriate sizes for preparing biochar. The biochar was prepared using a thermochemical process called pyrolysis, which occurred at a high temperature of 550°C in an oxygen-free environment. Subsequently, the biochar underwent grinding and sieving procedures using a mill to obtain uniformly sized particles that were less than 2 mm in diameter [12].

2.4 Preparation of new ration

Standard pellet diet was prepared by passing it through a 2 mm sieve to ensure uniformity in the size of the grains, facilitating effective blending. Subsequently, biochar was added to the prepared ration at a level of 1%.

2.5 Methane gas emissions

The study was conducted within the Kuwait Institute for Scientific Research facilities. Methane emissions quantification was carried out using respiration chambers and methodological and conceptual frameworks that were previously developed during the 2011 and 2012 greenhouse gas experiments [7, 13].

Fourteen female Naemi sheep, aged between 10 and 12 months, were kept in respiration chambers. Following the outlined protocol in [7], methane emissions were measured.

Before collection, each sheep underwent a 21-day acclimatization period, during which time it was offered a basal diet. After acclimatization, methane emissions were recorded over nine weeks.

The sheep received food twice a day in equal quantities at staggered times: 5:00 a.m. (hour 0) and 11:00 a.m. (hour 6). The subjects were randomly assigned to two groups of seven individuals each to ensure equal allocation.

2.6 Statistical analysis

The results obtained from the field experiment were analyzed using GraphPad Prism 10 software. The treatment values were compared against the control group values. Differences in variance were evaluated using one-way ANOVA, where statistical significance was set at the $P < 0.05$ level.

3. Results

3.1 Standard diet prepared

The standard diet's overall composition consisted of 70% concentrates and 30% roughage. Details regarding the formulation of the standard diet are provided in **Figure 1**.

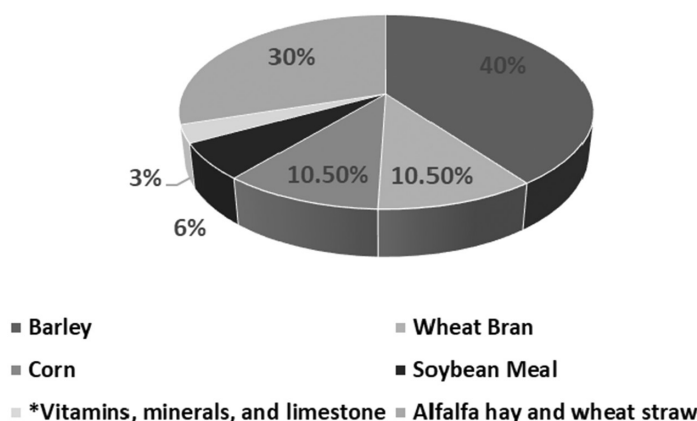


Figure 1.

*The experimental basal ration, tailored to the specific nutritional needs of Naeemi sheep and expressed on a dry matter basis, consists of the following ingredients: barley (40%), wheat bran (10.5%), corn (10.5%), soybean meal (6%), and a premix containing vitamins, minerals, and limestone (3%), as well as a mixture of alfalfa hay and wheat straw (30%). *the premix composition includes phosphorus (5%), calcium (18%), sodium (5%), potassium (5%), manganese dioxide (500 mg/kg), cobalt sulfate (100 mg/kg), zinc oxide (2000 mg/kg), calcium iodate (125 mg/kg), sodium selenate (10 mg/kg), vitamin a (400,000 IU/kg), vitamin D₃ (100,000 IU/kg), and alpha-tocopherol (400 iu/kg) (source: developed by the authors).*

3.2 Patterns of growth, development, and weight gain

Results showed that adding biochar to the basal diet significantly ($P < 0.003$) enhanced body live weight, along with height at withers (HAW), body length, and heart girth (HG) or chest circumference in Naeemi sheep compared with the control.

Mean body live weight for sheep on the basal diet with biochar addition was 64.19 kg, compared with 59.81 kg for the control (**Figure 2**).

Significant progress was also noticed in growth parameters within the biochar application treatment plot; these sheep measured 80.49 cm in body length, 82.95 cm in heart girth, and 100.39 cm in height at the withers.

On the other hand, the corresponding measurements for the control were 77.23 cm, 79.99 cm, and 96.31 cm (**Figure 2**).

3.3 Body condition score (BCS)

Animals following a basic feeding protocol with biochar supplementation showed a significantly higher average body condition score (BCS) of 3.5, compared to a control BCS of 2.76.

Numerous studies have irrefutably demonstrated that adding biochar to the diets of sheep has a positive effect on fat deposition. Biochar, a carbon-rich solid material made from organic matter, has been found to be effective in increasing nutrient utilization efficiency along with feeding efficiency in ruminant species.

When incorporated into the feeding plan for these animals, biochar can modify digestive processes, ultimately improving nutrient absorption efficiency, especially for fats, which, in turn, promotes increased fat deposition within the animals (**Figure 2**).

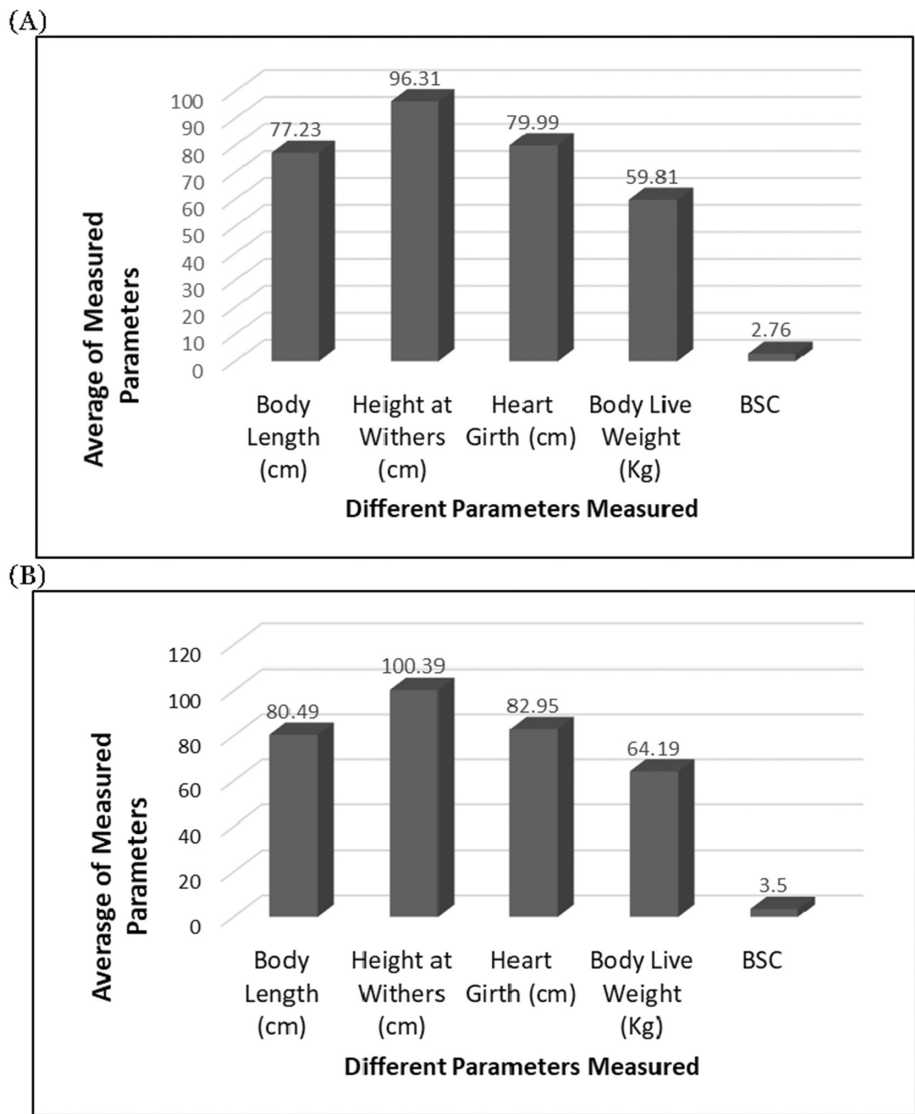


Figure 2.
 Average body length, height at withers, heart girth, body live weight, and body condition score (BCS) of Naeemi sheep fed (a) a basal ration (control) and (b) a basal ration supplemented with biochar during the feeding trial. Values are expressed as means (source: developed by the authors).

3.4 Methane emission control

Table 1 shows that on Day-0, immediately before biochar was introduced into the diets of the subjects, the baseline methane emission rate observed across all participants under the two experimental conditions was 1,070 ppm.

The experimental group, whose diets were supplemented with biochar, had a significantly lower methane production rate ($P < 0.05$), recording declines of 71.52% and 74.24% in weeks 1 and 9, respectively, compared to the control group.

Duration in weeks	Standard diet (Control)	Standard diet + Biochar	% of CH ₄ reduction
Day-0	1,070 ± 0.21	1070 ± 0.21	-
1	1,072 ± 0.02	625 ± 0.03	71.52
2	1,074 ± 0.01	626 ± 0.04	71.57
3	1,076 ± 0.14	630 ± 0.98	70.79
4	1,072 ± 0.22	620 ± 0.19	72.90
5	1,074 ± 0.31	622 ± 0.23	72.67
6	1,077 ± 0.10	624 ± 0.87	72.59
7	1,078 ± 0.23	625 ± 0.67	72.48
8	1,077 ± 0.02	630 ± 0.32	70.95
9	1,096 ± 0.03	629 ± 0.04	74.24

Table 1. Methane (CH₄) gas production (ppm) in Naeemi sheep fed a basal ration (control) or a basal ration supplemented with biochar over a 9 week feeding period. Values are presented as mean ± SEM, with percentage CH₄ reduction calculated relative to the control group (source: developed by the authors).

During the experimental period, the mean SMC in the control group did not change at all, remaining at 1,076.6 ppm, with recorded fluctuations between 1,070 and 1,096 ppm, and no statistically significant differences were noted over time ($P > 0.05$).

In contrast, the experimental group exhibited a significant and sustained decline in methane emissions after the introduction of biochar. Emission rates consistently decreased from a baseline of 1070 ppm on Day 0 to an average of 629 ppm by Week 9. The decline was found to be statistically significant ($P < 0.05$) compared to both the control group and the baseline measurements, ranging from 70.79% to 74.24%.

3.5 Total feed consumption

(**Figure 3**) presents the average feed conversion ratio (FCR) for all animal groups used in the study (kg/2 s). FCR is a key determinant of feeding efficiency, particularly in assessing study (kg/2 week). FCR is a key determinant of feeding efficiency, especially with the ability of livestock to transform feed into body mass, such as meat.

FCR for the control group was observed to be 16.87 ± 0.92 . The biochar-supplemented group, however, showed a significantly higher FCR value of 18.99 ± 0.68 .

The purpose of the present study was to design a novel dietary regimen for sheep to reduce methane emissions caused by ruminant fermentation. Observational data showed that Naeemi sheep treated with a supplemented diet of biochar exhibited significantly enhanced body live weight and several growth parameters, including body length, height at withers, heart girth, and body condition score, compared with the control group. These observations align with findings reported in earlier studies [8].

A noteworthy feature of the present study's outcomes is the evidence of enhanced feed efficiency resulting from supplementation with biochar.

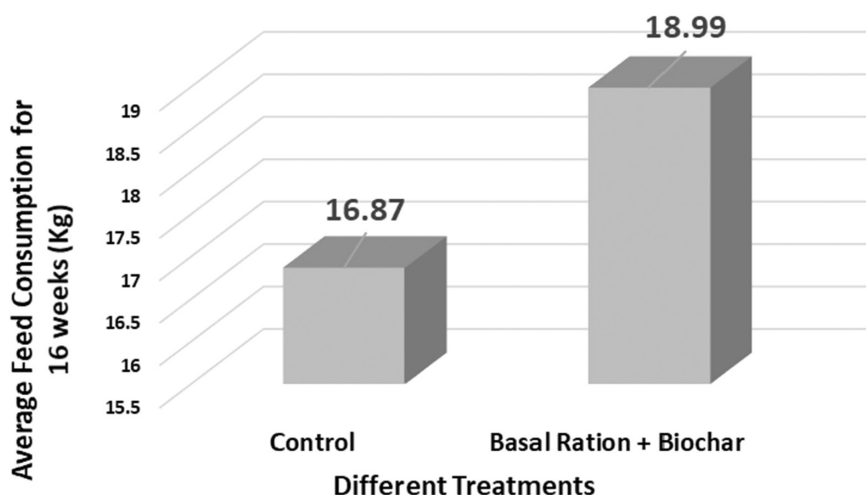


Figure 3.
 The average feed consumption ratio (kg) of Naeemi sheep across a 16-week feeding period was determined when they were fed a basal diet (control) or a basal diet supplemented with biochar. The biochar supplementation of the feed increased feed intake from 16.87 to 18.99 kg/2 weeks in comparison to the control feeding regimen (source: developed by the authors).

Biochar improves the absorptive capacity and digestibility of essential nutrients, resulting in increased assimilation and greater availability of these nutrients. Moreover, biochar acts as a binder, thereby reducing excretory losses and enhancing nutrient utilization. Existing literature substantiates a connection between biochar application and increased weight gain, as well as overall growth in livestock [14].

4. Discussion

The major objective of this study was to develop a modified feeding regime for sheep to substantially reduce methane emissions during ruminant digestion. The experiment involved two different groups of Naeemi sheep: one group was fed a biochar-enriched diet, and the other group was fed a standard diet.

The results showed that the sheep from the biochar-enriched feeding regime exhibited significantly greater body mass and growth parameters, including body length, height at the withers, and heart girth, compared to the control group. These findings support previous observations reported by [15]. Such improvements are largely attributed to biochar's ability to enhance the breakdown and assimilation of nutrients, thereby optimizing feeding efficiency.

In addition, biochar helps to decrease nutrient leaching, which optimizes feed utilization, leading to increased weight gain and growth in livestock, as reported in previous research [15]. Moreover, biochar has been associated with improved gastrointestinal health [16, 17], as it enhances the proliferation of beneficial gut microorganisms, stimulates immunity, and reduces the occurrence of various diseases.

Importantly, biochar can adsorb harmful contaminants, such as toxins, heavy metals, and mycotoxins in the gastrointestinal tract before they can enter the bloodstream, thus leading to better health in livestock.

It is essential to recognize that excessive use of biochar can restrict root penetration and result in variations in nutrient availability, as reported by some previous studies [18, 19].

4.1 The effects of incorporating biochar in livestock feeds.

Earlier studies have shown that including bamboo biochar at a dose of 1 g/kg of live weight produces significant results [6, 20, 21]. Goats fed diets supplemented with biochar experienced an increase in crude protein intake and improved nitrogen absorption, leading to lower nitrogen excretion through urine and feces.

Additionally, the goats in the study showed a notable rise in their average daily weight gain, increasing by 53 grams from the 44 grams recorded in the control group. To gain further insights into this phenomenon, the study used a controlled feeding regime primarily involving tannin-rich *Acacia mangium* leaves to determine if digestive efficiency was improved through the combined effects of tannins and biochar, thereby increasing the availability of protein and dry matter for better nutritional uptake.

In a parallel study involving 12 groups of goats, researchers compared growth performance between scenarios with a 1% biochar addition to a standard mixture of *Bauhinia acuminata* leaves – known for their high tannin content – and scenarios without biochar. The addition of biochar in this setup resulted in better nutrient absorption, leading to a 27% increase in daily weight gain over the 100-day feeding period. However, the results presented here show some inconsistencies regarding the reported improvements.

Furthermore, in another specific study on goats fed diets with 1.5% activated coconut biochar as well as with 3%, no variation in feeding rates or microbial community compositions was observed when compared with control animals [21]. Such contradictory results show that the effectiveness of biochar supplementation is highly dependent on numerous factors, such as the type of livestock involved, the nutrient composition of the feeds used, the choice of biochar type, the dosages applied, and the experimental conditions employed.

The impact of biochar addition on the feed conversion ratio (FCR) shows variation with respect to species. Some research findings suggest a reduction in the FCR due to biochar addition. For instance, in broiler chickens, biochar addition increased feed conversion efficiency while simultaneously inducing a reduction in overall food intake, thereby producing a better FCR ratio [8]. Similarly, the addition of biochar to breeding rabbit diets resulted in enhanced feeding efficiency, accompanied by a decline in food intake [14]. Conversely, other studies reported negligible or no significant effects.

Studies involving monogastric animals often show different results. In 2025, a study on mature pigs treated with commercial feed supplemented with 2% biochar found no statistically significant differences in average daily feed intake (ADFI), average daily weight gain (ADWG), and feed conversion ratio (G:F); however, there was a notable improvement in crude fiber digestion [22].

A more detailed study conducted in 2025 also yielded varied results, indicating that the effect of biochar on feed efficiency and growth performance is not always positive for monogastric animals [23]. In contrast, results for ruminant animals have generally been more promising.

In a highly controlled crossover trial with lactating dairy cows in late 2024, feeding 1% biochar had no significant effect on feed intake, milk yield, quality, or enteric methane emissions. Conversely, a meta-review in 2025 reported that adding biochar to various livestock species, such as fattening bulls, pigs, broilers, and sheep, consistently improved feed conversion efficiency, digestive function, and body weight gain, while also reducing enteric methane emissions (by up to 30–40% in cattle) [24, 25].

These contrasting findings highlight a key point: the effectiveness of biochar supplementation largely depends on animal species, baseline diet and health, biochar source and dosage, and experimental conditions. While responses in monogastric animals vary, outcomes in ruminants seem more favorable but are still context-dependent. [25]

4.2 Effect of adding biochar to sheep diets on body conditions

The animals that received biochar-supplemented diets had a significantly higher mean body condition score (BCS) value of 3.5, as opposed to a BCS value of 2.76 among the control animals. These findings align with those reported in [26], which solidify the health-improving effects of biochar on the deposition of adipose tissue in ruminant hosts. Biochar, defined as a substance derived from biomass that is similar to charcoal, has proven effective in improving nutrient utilization efficiency and enhancing feed conversion ratios in feedlot populations of livestock.

In sheep nutrition, biochar supplementation has been shown to influence digestive mechanisms, thereby enhancing nutrient absorption, particularly lipids. This increased nutrient uptake is thought to contribute to the enhanced fat deposition observed in biochar-fed animals. The current study supports these findings.

For example, a previous study conducted on Awassi lambs receiving a 1% biochar dosage for 90 days showed improvements in carcass fat percentage and intramuscular fat deposition when compared to control groups, without showing any adverse effects on measures of meat quality [17, 27].

Another study carried out on Barki sheep showed that adding bamboo-derived biochar to their diet significantly improved fat deposition in adipose tissue and seemed to maximize energy use efficiency [16, 26]. Though the precise mechanisms of action are unknown, more recent studies have noted several contributing factors: enhanced nutrient assimilation, favorable changes in rumen populations of microbes (fostering bacteria with lipid-synthesizing capability), and improved barrier integrity of the intestine.

Overall, across multiple experiments with lambs and mature sheep fed varying biochar levels (typically 0.5–2% of diet dry matter), a consistent trend emerges: increased fat deposition and carcass lipid content, supporting biochar's potential role as a feed additive to improve body condition and productivity in small ruminants.

4.3 Minimization of greenhouse gas emissions

The information contained in **Table 1** shows a significant reduction in methane gas emissions after adding biochar to livestock feeding diets. A baseline measurement of methane emissions was initially recorded at 1070 ppm in week 0.

From week 1 onwards, methane emissions were reduced in all treatment groups (**Table 1**), with reductions ranging from 70.79% to 74.24% when compared to the entire field experiment and the control plot. These results are consistent with previous studies carried out by [28], which illustrate that biochar is an effective measure for reducing methane emissions from livestock because it is thought to suppress methanogenic activity by altering gut microbial communities.

Findings of the current study concur with recent research indicating the potential of biochar inclusion to substantially reduce enteric methane emissions from livestock. For example, an extensive review concluded that adding biochar to animal diets improved digestion and microbial action, simultaneously achieving considerable reductions in methane production of between 30% and 40% in cattle, with significant reductions observed in goats and poultry as well.

Furthermore, another experimental study undertaken in 2025 showed that biochar improved nutrient use, augmented feeding efficiency, and substantially reduced emissions of methane among diverse livestock tested [28]. However, variability cannot be overstated, as illustrated by a Swiss crossover study involving lactating cows given 1% biochar, which found no significant effects on methane outputs, milk yield, digestibility, and performance generally.

These inconclusive outcomes indicate that the effects of biochar inclusion on mitigating methane emissions are moderated by several variables, including the quality and type of biochar, the dosing rate used, dietary composition, animal species, and the environmental conditions under which they are kept.

Aside from its environmental importance, the use of biochar in lamb feeding has been linked to increased feed digestibility. Although this factor has been relatively less emphasized in recent studies, several studies have confirmed this benefit. A recent review once again linked biochar with improved feed digestion and nutrient absorption [28].

In agreement, a 2023 trial showed that the addition of corncob-based biochar led to a significant rise in dry matter intake, crude protein intake, and dry matter digestibility compared to control groups. Together, these studies highlight the twin merits of using biochar in feeding small ruminants: better environmental sustainability and increased nutritional effectiveness [29].

5. Conclusions

This study clarifies that the integration of sterile date-palm biochar into the feed of Naeemi sheep provides widespread benefits related to ecological sustainability and animal productivity. Specifically, the incorporation of 1% biochar into feed led to a significant reduction in enteric methane emissions, decreasing in the range of 70.79% to 74.24% across a nine-week feeding experiment, thus validating its role as a tool to curb greenhouse gas emissions from ruminant livestock.

Besides this specific environmental advantage, biochar-supplemented sheep demonstrated increased body weight, superior growth parameters, better body condition scores, and an increased feed conversion ratio in comparison to control groups. The results can be attributed to the high surface area and inherent cation-exchange capacity of biochar, which allow enhanced absorption of nutrients, increased efficiency of rumen fermentation, and favorable bacterial population substitutions leading to methanogenesis attenuation.

The discovery aligns with previous research examining the role of biochar in enhancing nutrient retention, increasing back fat deposition, and improving feed efficiency in ruminant species. Even though the results show promising implications, variability across previous research indicates the manifestation of varied factors, including types of biochar, biochar particle sizes, rates of use, types of diets, and the species-specific nature of the digestive system. More studies must be conducted to define optimal rates of use, desirable levels of integration, and to investigate wider implications for animal welfare, manure quality, and general agronomic productivity within the realm of integrated farming environments.

The outcome of the study supports the use of biochar as a multi-purpose feed supplement that ensures climate-resilient livestock production, improved animal performance, and, consequently, the creation of stronger and more resilient agricultural systems.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 3

Primary Phytochemicals in Under-looked Edible Plant Species: Improving Sheep Health, Productivity and Meat Quality Using Succulent Plants

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Abstract

The global demand for high-quality, healthy, and safe meat and meat products continues to escalate rapidly, as unhealthy foods are currently the major cause of long-term health risks, mainly cancer and cardiovascular diseases, especially in adults. For this reason, the use of synthetic antibiotics, growth promoters, and pharmaceuticals to enhance performance and the immune system is highly discouraged in livestock production. Alternatively, dietary phytochemicals have been heavily recommended as natural agents for enhancing the immune system, microbial fermentation, antioxidant activity, muscle development, and metabolism in ruminant animals. The supplementation of phytochemical-rich plant materials in diets for small ruminants, such as sheep, could aid in improving overall health, nutritional value, and safe consumption while preventing methane emissions, and lipid peroxidation of meat, milk, and other by-products. However, there is little information reported on the potential of phytochemicals present in under-looked plant feed ingredient inclusion, mainly the edible succulent shrubs, in sheep diets. Hence, this chapter uncovers functional phytonutrients present in edible succulent shrubs as potential supplements in sheep diets. Thus, the nutritional value, especially the type and concentration of secondary metabolites present in selected succulent plants, is outlined and compared. Thereafter, the potential benefits of various functional phytochemicals identified in selected edible succulents on the health, performance, methane reduction, and meat quality of sheep are thoroughly discussed.

Keywords: antioxidant activity, bioactive compounds, drought-resistant plants, methane emission, succulent shrubs, sheep farming

1. Introduction

Ruminant meat, including lamb and mutton, generates more gross income in most countries than meat from other livestock species. According to Jayathilakan et al. [1] and Alao et al. [2], the overall edible and non-edible by-products of sheep constitute 68% of the total live weight, whereas edible parts make up 55% of the whole products. Hence, meat, milk, and wool produced from sheep significantly contribute to the livestock industry and global protein demand in response to the rapid escalation of human population growth. In addition, the drastic changes in consumer attitudes toward a healthy lifestyle have led to an increase in demand for fresh, nutritious, functional, and biofriendly foods [3, 4]. Thus, it is important to prioritize the health of sheep by considering natural-based remedies and reducing the overreliance on drug medications and vaccines, which ultimately affect human health after consuming sheep meat, milk, and other products. Dietary phytonutrients or bioactive compounds present in plant-based feed ingredients, including various trees, shrubs, grasses, and herbs, are functional essential nutrients that could be vital in maintaining good health when included in diets for livestock [5–7].

These vital secondary metabolites are widely documented to possess antioxidant, antimicrobial, antihypertensive, anti-inflammatory, antiviral, antidiabetic, antiprotozoal, antidiarrheal, anthelmintic, antiallergic, antispasmodic, and chemoprotective properties in living organisms, mainly plants and animals [4, 8–13]. In plants, they form part of the nutrients or organic compounds responsible for growth, development, protection, and efficient metabolism, whereas they have a tremendous influence on the immune system, as well as metabolic and antioxidant activity in both humans and livestock [14].

Of the 100,000 phytochemicals identified in edible plants, only about 150 have been studied extensively in common plant foods such as herbs, fruits, and vegetables for human and animal consumption [12]. Whereas limited studies have been conducted on phytochemicals present in drought-resistant plants such as succulent shrubs, particularly for inclusion in livestock diets. Common edible succulents found in arid and semi-arid regions, including *Opuntia ficus-indica*, *Cylindropuntia* spp., *Echinocereus* spp., *Hoodia gordonii*, *Ferocactus* spp., *Carnegiea gigantea*, *Hylocereus undatus*, *Aloe barbadensis*, *Atriplex nummularia*, *Salicornia* spp., *Portulaca oleracea*, and *Portulacaria afra*, are also rich in phytochemicals. Although the majority of these edible shrubs are mainly explored in pharmaceutical and medicinal fields to improve human health [12], they could also play a significant role as animal feed additives or supplements to provide essential phytochemicals for livestock, such as sheep, during droughts and feed shortages due to climate change [6, 15–17]. However, factors influencing the availability, concentration, and effectiveness of phytonutrients present in edible succulent plants include plant type, moisture content, proximal composition, processing, and storage. Additionally, the health benefits of dietary phytonutrients are mainly dependent on their yield, purity, and structural ability [13]. Hence, this chapter intends to explore and summarize the nutritional value of selected succulent plants and their potential phytochemical effects on health and sheep productivity.

2. Methodology

The information from this review was obtained from published articles available on Google Scholar, Web of Science, Scopus, ResearchGate, ScienceDirect, and PubMed databases. The main keywords used for the current literature search were dietary phytochemicals, phytochemicals, phytochemicals, phytonutrients, bioactive compounds, phenolic compounds, polyphenols, succulent plants, and drought-resistant crops. The search strategy focused solely on original research and review articles written in English. Almost all articles published after 2010 were included in this search; however, nine significant research articles published before 2010 were also incorporated. Additionally, an analysis was conducted using the keywords employed in the literature search to examine plant phytonutrients and their applications in human and livestock studies using VOS Viewer [18].

3. Results and discussion

3.1 Nutritional composition of selected succulent plants

3.1.1 Proximate composition

Proximate composition is vital in determining the nutritional value of animal feed ingredients by evaluating the concentrations of macronutrients, mainly protein and energy, prior to supplementation in the diets of livestock such as sheep [19]. As shown in Figures 1–12 and 1–12 embedded in **Tables 1** and **2**, the selected edible succulent plants contain high moisture contents of up to 93%, which is expected since drought-resistant plants such as cactus pulps and fruits are known to retain high moisture content. Hence, they could be important for sheep production by providing additional water for livestock in areas adversely influenced by droughts and water shortages due to elevated temperatures caused by climate change [20]. Although protein levels are very low in most succulent shrubs, *Atriplex nummularia* leaves, as well as leaves and stems of *Portulaca oleracea*, were reported to contain higher crude protein, which could be vital in improving the protein content in diets, thus contributing to muscle development when included in sheep diets. Nonetheless, seeds and fruits of *Opuntia ficus-indica* and *Hylocereus* spp. have the lowest crude protein compared to other selected succulent plants. Fat content is reported to be higher in *Carnegiea gigantea* seeds and *Atriplex nummularia* leaves, which could act as an additional energy source when supplemented in sheep diets, while the lowest fat content is found in *Hylocereus* spp. fruits, as well as in leaves and stems of *Portulaca oleracea*. Ash levels are high in flowers, fruits, and seeds of *Cylindropuntia* spp., followed by *Salicornia* spp. leaves, which indicates higher mineral contents such as calcium that could be vital for bone development when included in sheep diets. However, the lowest ash content is reported in *Hylocereus* spp. fruits and *Atriplex nummularia* leaves.

Higher crude fiber is reported in the flowers, fruits, and seeds of *Cylindropuntia* spp. and *Aloe barbadensis* leaves, which could be important not only for improving digestibility but also for promoting the growth and development of beneficial microbes in the rumen and abomasal compartments of sheep, thus improving gut health. Nevertheless, the lowest fiber content is found in *Hylocereus* spp. fruits as well

Plant species	Plant part	Moisture	Crude protein	Fat/lipids	Ash	Crude fiber	Carbohydrates	Energy (kcal/100 g)	References
Prickly pear cactus (<i>Opuntia ficus-indica</i>)	Seed, peels, and fruit	80.17–87.07	0.60–4.78	0.40–4.88	1.27–8.00	0.96–12.47	92.57	-	[21–23]
Rosecactus (<i>Cylindropuntia</i> spp.)	Flowers and fruits	65.31–79.21	6.22–10.93	1.96–5.42	4.46–24.44	8.47–47.77	67.44	348.05	[24, 25]
Hedgehog Cactus (<i>Echinocereus</i> spp.)	Flowers	-	13.64	2.18	16.83	11.52	55.83	320.51	[24]
Bushman's hat (<i>Hoodia gordonii</i>)	Pulp	-	5.90	-	-	-	-	-	[26]
Fishhook barrel cactus (<i>Ferocactus</i> spp.)	Pulp, flower and fruit	91.70	1.20–8.20	1.30–5.80	1.60–8.17	1.10–20.00	18.80–83.04	4.17	[27–30]
Saguaro Cactus (<i>Carnegiea gigantea</i>)	Seeds	-	20.30	29.40	3.40	1.80	-	-	[31]
Dragon Fruit (<i>Hylocereus undatus</i>)	Fruit	80.00–86.12	0.40–2.20	0.40–0.67	0.54–0.71	0.03–0.27	8.50–11.05	53.89	[32, 33]
Aloe vera (<i>Aloe barbadensis</i>)	Pulp	11.7–91.12	4.73–8.75	-	2.36–16.88	7.84–73.35	52.0–73.08	238.00	[34–37]
Oldman Saltbush (<i>Atriplex nummularia</i>)	Leaves	-	16.70–25.20	18.50–34.10	1.00–1.60	-	-	-	[38]
Glasswort (<i>Salicornia</i> spp.)	Leaves	81.79–88.42	1.54–13.82	0.37–1.37	4.36–31.24	0.83	4.48–14.39	-	[39, 40]
Common purslane (<i>Portulaca oleracea</i>)	Leaves	88.4–91.00	1.30–44.35	0.10–0.15	1.89–23.29	-	1.45–7.60	16.00–47.00	[41]
Spekboom (<i>Portulacaria afra</i>)	Leaves	93.18	0.57–11.80	-	11.80	1.17–14.60	57.90	13.84	[42, 43]

Table 1. Proximate composition (% on a dry matter basis, unless stated otherwise) of under-looked edible succulent plant species.

Plant species	Plant part	Macro minerals					Micro minerals										Antinutrients and secondary metabolites								References
		Potassium	Calcium	Phosphorus	Sodium	Magnesium	Iron	Zinc	Copper	Manganese	Alkaloids	Flavonoids	Oxalates	Phytates	Saponins	Tannins	Total phenols	Total carotenoids							
Prickly pear cactus (<i>Opuntia ficus-indica</i>)	Seed and fruit	108.8–535.7	316.5–471.2	0.05–16,527.50	18.70	63.4–117.3	11.7–25.9	3.2–12.6	0.01–2.10	1.90–37.80	-	190–0.00	-	2590.00	-	1500.00	1500.00	2970.00–12,760.00	[21–23]						
Roseakaktus (<i>Cylindropuntia</i> spp.)	Flowers, fruits, and seeds	18.49–44.67	22.35–172.19	0.96–1.82	0.08–1.59	12.86	0.31–0.13	0.08–0.21	-	0.07–0.19	-	9.93	-	-	-	-	1.0–5.29	0.26	[24, 25, 52]						
Hedgehog Cactus (<i>Echinocereus</i> spp.)	Flowers and seeds	-	-	-	-	-	-	-	-	-	-	40.61–71.81	-	-	-	0.002–0.051	13.31–44.63	0.24	[24, 53]						
Bushman's hat (<i>Hoodia gordonii</i>)	Pulp	5.10	6.50	0.30	-	1.00	1.90	-	-	-	130.00	-	-	-	-	-	-	-	[26]						
Fishhook barrel cactus (<i>Ferocactus</i> spp.)	Fruit	50.29	-	-	-	9.73	2.71	0.11	0.20	0.08	-	-	-	-	-	-	-	-	[29]						
Saguaro Cactus (<i>Carnegiea gigantea</i>)	Seeds	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6.40	-	[31]						

Plant species	Plant part	Macro minerals					Micro minerals					Antinutrients and secondary metabolites							References
		Potassium	Calcium	Phosphorus	Sodium	Magnesium	Iron	Zinc	Copper	Manganese	Alkaloids	Flavonoids	Oxalates	Phytates	Saponins	Tannins	Total phenols	Total carotenoids	
Dragon Fruit (<i>Hylocereus</i> spp.)	Fruit	175.93–	6.66–	14.66–		23.99–	0.20–	0.16–	0.02	0.05–	-	4.82–	-	-	-	-	126.03–	-	[32,
		294.23	8.50	22.50		33.31	1.90	0.28		0.08		25.68					139.39		33]
Aloe vera (<i>Aloe</i> <i>barbadensis</i>)	Pulp	1.06		0.67	0.52	0.03	0.003	0.007	0.002	0.013	2470.00–	-	450.00	-	5,650–	160.00–	230.00	-	[34]
											31,070.00				10,670.00	225,660.00			
Oldman Saltbush (<i>Atriplex</i> <i>nummularia</i>)	Leaves	1.68–	0.63–	0.09–	0.21–	0.55–	0.25–	39.70	20.00–	59.00–	-	-	2810.00–	-	-	-	-	-	[38,
		6.06	2.49	0.28	6.66	0.84	0.51		26.00	89.00			4490.00						54, 55]
Glasswort (<i>Salicornia</i> spp.)	Leaves	1.76	0.62	0.18	9.98	1.18	0.01	0.004	0.91	-	-	-	-	-	-	-	-	-	[39]
Common pur- slane (<i>Portulaca</i> <i>oleracea</i>)	Leaves	0.18–	0.11–	0.26	-	-	0.01–	-	2.47–	-	-	-	-	-	-	-	-	-	[56]
		0.58	0.27	-1.19			0.04		9.21										
Spaghboom (<i>Portulacaria</i> <i>afra</i>)	Leaves	-	-	-	-	-	-	-	-	-	-	107.42–	-	-	-	-	1038.18–	-	[57, 58]
												901.30					9521.01		

Table 2.
 Minerals and secondary metabolites (mg/g) of selected under-looked edible succulent plants.

as the leaves and stems of *Salicornia* spp. Carbohydrates are predominantly high in the seeds and fruits of *Opuntia ficus-indica*, followed by *Ferocactus* spp. fruits, which could provide a significant amount of additional energy when included in sheep diets, whereas the lowest carbohydrate content is reported in *Portulaca oleracea* leaves and stems. However, higher energy levels are present in *Cylindropuntia* spp. flowers, fruits, and seeds, which could be crucial in sheep production, especially in arid and semi-arid areas that experience feed shortages due to high feed costs and the effects of climate change. The lowest energy content, however, is found in the fruits of *Ferocactus* spp. as well as the leaves and stems of *Portulacaria afra*. Thus, higher contents of crude protein (in *Portulaca oleracea* and *Atriplex nummularia*), ash (*Cylindropuntia* spp. and *Salicornia* spp.), crude fiber (in *Cylindropuntia* spp. and *Aloe barbadensis*), and carbohydrates (*Opuntia ficus-indica* and *Ferocactus* spp.) compared to other succulent plants indicate that they could potentially play a significant role in improving the nutrient contents of low-quality diets provided to sheep during hot, dry seasons.

3.1.2 Macro and micro-mineral contents

Minerals are important for growth, health, and productivity in ruminants, including sheep and goats [44]. As depicted in **Table 2**, seeds and fruits of *Opuntia ficus-indica* and *Hylocereus* spp. fruits have elevated potassium levels, respectively, while the lowest levels are found in the leaves and stems of *Portulaca oleracea* compared to other selected edible succulent shrubs. Similarly, calcium is observed to be higher in *Opuntia ficus-indica* seeds and fruits but lowest in the leaves and stems of *Portulaca oleracea*, *Salicornia* spp., and *Atriplex nummularia* leaves, respectively. Phosphorus is higher in the seeds and fruits of *Opuntia ficus-indica* while lowest in *Aloe barbadensis* pulps, followed by *Atriplex nummularia* leaves as well as the leaves and stems of *Salicornia* spp. and *Portulaca oleracea*. Sodium is also elevated in *Opuntia ficus-indica* seeds and fruits but lowest in the flowers, fruits, and seeds of *Cylindropuntia* spp. and *Aloe barbadensis* pulps. The seeds and fruits of *Opuntia ficus-indica* and *Hylocereus* spp. fruits, respectively, have the highest magnesium levels, while it is lowest in *Aloe barbadensis* pulps and *Atriplex nummularia* leaves compared to other selected edible succulents. In terms of micro minerals, iron is higher in *Opuntia ficus-indica* seeds and fruits but lowest in *Aloe barbadensis* pulps, followed by the leaves and stems of *Salicornia* spp. and *Portulaca oleracea*. Elevated zinc is observed in the leaves of *Atriplex nummularia* but lowest in *Aloe barbadensis* pulps, followed by the leaves and stems of *Salicornia* spp. However, copper and manganese, on the other hand, are reported to be higher in *Atriplex nummularia* leaves, while lowest in *Hylocereus* spp. fruits and *Aloe barbadensis* pulps, respectively. Thus, the higher mineral contents observed in *Opuntia ficus-indica* compared to other selected succulents indicate that the seeds and fruits of cactus species can be beneficial in providing both essential macro and micro minerals responsible for enhancing health and productivity when supplemented in sheep diets.

3.1.3 Secondary metabolites

Bioactive compounds have been well-studied in various fields, including biology, agriculture, biotechnology, and phytomedicine, due to their significant contribution to the health of humans and domesticated animals. These bioactive compounds

essentially function as pigments that contribute to the color, odor, flavor, and texture of most plant foods [5]. According to Guan et al. [12], phenolic acids, tannins, and flavonoids are the most common classes of phytochemicals found in various plant species. However, in human studies, phenols such as carotenoids, polyphenols, isoprenoids, phytosterols, tannins, saponins, dietary fibers, and polysaccharides have been widely documented [13]. On the other hand, various livestock diets constitute alkaloids, flavonoids, saponins, tannins, oxalates, phytates, and essential oils as the main functional groups of phytochemicals to provide health benefits [10, 45, 46]. **Figure 1** indicates the following: The green and purple colors represent studies where phytonutrients were detected in various fruits, vegetables, herbs, and their extracts. Most of these studies focused on different types of flavonoids, polyphenols, carotenoids, alkaloids, and tannins, and their antioxidant, antimicrobial, antihypertensive, and anti-inflammatory properties when provided orally or in diets for humans and livestock. However, the blue and red colors of the web indicate a few studies published mostly on enzyme and antioxidant activities of phytochemicals present in spices, herbs, and some fruits.

As shown in **Table 2**, alkaloids were only reported in *Hoodia gordonii* and *Aloe barbadensis* pulps. The steroidal glycoside alkaloids, known as P57 in *Hoodia gordonii*, act as appetite suppressants that could aid in improving feed and water use efficiencies in ruminants such as sheep. Flavonoids detected in several succulent plants were abundant in the leaves and stems of *Portulacaria afra*, followed by *Opuntia ficus-indica* seeds and fruits, as well as pulps, flowers, and fruits of *Echinocereus* spp., while lower in the flowers, fruits, and seeds of *Cylindropuntia* spp.

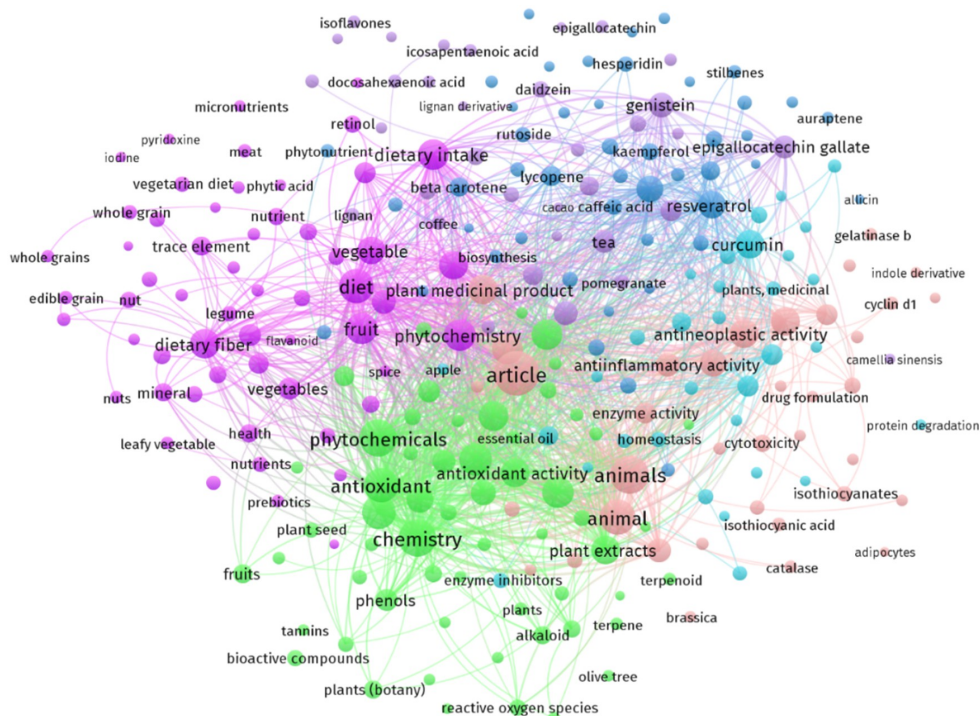


Figure 1. Schematic representation of plant phytonutrients and their application in human and livestock studies [18].

Dietary intake of flavonoids, such as isoflavone, has antioxidant potential associated with reducing incidents of heart-related diseases [47, 48].

However, antinutrient oxalates were only detected in *Aloe barbadensis* pulps and *Atriplex nummularia* leaves, hence, their supplementation at high concentrations could adversely affect bone health and growth by reducing calcium absorption in the rumen and abomasum of sheep. Dietary phytates, which also act as antinutritional factors by reducing the bioavailability of various minerals, including calcium, iron, and zinc, were only reported in *Opuntia ficus-indica* seeds and fruits, while saponins were observed in *Aloe barbadensis* pulps. Similar to oxalates and phytates, excessive saponins in diets also possess anti nutrient properties as they reduce nutrient absorption and could ultimately cause bloating in ruminants such as sheep. Low levels of tannins, on the other hand, are reported in *Opuntia ficus-indica* seeds and fruits, followed by *Aloe barbadensis* pulps and *Echinocereus* spp. pulps, flowers, and fruits. Lower tannin levels could aid in reducing methane production and emission by ruminants and could also protect proteins from degradation in the rumen of sheep [49].

Total phenols were higher in *Portulacaria afra* leaves and stems but lowest in the flowers, fruits, and seeds of *Cylindropuntia* spp. Functional phenolic acids have been reported to possess antibacterial, antitumor, and anti-inflammatory properties that could be beneficial for livestock, particularly ruminants such as sheep [50]. Total carotenoids were only detected in the seeds and fruits of *Opuntia ficus-indica*, followed by the flowers, fruits, and seeds of *Cylindropuntia* spp. as well as *Echinocereus* spp., but were higher in *Opuntia ficus-indica* while significantly lower in *Cylindropuntia* spp. and *Echinocereus* spp. According to Prasanthi et al. [19], carotenoids and polyphenols possess antioxidant activities responsible for regulating the immune system and preventing cardiovascular, cancer, and age-related diseases, hence, they could be vital in improving the immunity of ruminant animals such as sheep when included in their diets [51]. Indeed, dietary supplementation of drought-resistant plants, particularly *Opuntia ficus-indica* and *Aloe barbadensis* pulps, could play a significant role in sheep production as sources of essential phytonutrients.

3.2 Potential benefits of dietary phytonutrients from succulent plants in sheep production

3.2.1 Health, growth, and reproductive performance

The diet remains one of the paramount factors influencing livestock health and performance [4]. This is mainly due to the positive effects of phytochemicals that are abundant in plant feed ingredients, including succulent plants used as supplements during drought seasons. Tannins, particularly condensed tannins detected in *Opuntia ficus-indica*, *Echinocereus* spp., and *Aloe barbadensis*, could improve sheep performance by modifying and stabilizing the gut microbiota, destroying harmful microbes while enhancing the growth of beneficial microbes such as bacteria through antimicrobial and antioxidant activities [59, 60]. Lillehoj et al. [10] also emphasized that phytochemicals incorporated into diets significantly alter the gut microbiome of ruminant animals, ultimately influencing the digestion and absorption of nutrients as well as various metabolic pathways. Hence, improving intestinal health and functioning while inhibiting intestinal diseases [12]. Vast literature also indicates that phytochemicals, particularly flavonoids, tannins, and terpenoids found in the

seeds, leaves, and fruits of *Opuntia ficus-indica* and *Aloe barbadensis*, as well as in banana leaves and peels, exhibit antimicrobial and anthelmintic properties essential for controlling internal parasites in ruminant animals, especially sheep and goats [45, 61–63]. Hence, dietary phytonutrients help inhibit enteric pathogens while improving health and growth performance [10]. Moreover, the utilization of flavonoids (catechins and anthocyanins) in *Opuntia ficus-indica*, *Cylindropuntia* spp., *Echinocereus* spp., *Hylocereus* spp., and *Portulacaria afra*, which are also found in mango (*Mangifera indica*) peels, could act as natural antioxidants in sheep diets and those of cattle [64, 65]. Similarly, succulent phytochemicals such as flavonoids (quercetin and kaempferol) and organo-sulphur compounds (cepaenes and thiosulfates), also found in onion extracts used as a feed additive, serve as growth promoters to improve weight gain and feed efficiency in both ruminant and monogastric animals [15]. Additionally, a small amount of dried cabbage could be included in ruminant diets as a source of flavonoids that possess antioxidant properties, aiding in boosting immune response and preventing diseases that could compromise their health and performance [66, 67].

3.2.2 Milk quality and production

Dietary supplementation with mainly bioflavonoids abundant in *Portulacaria afra* and *Opuntia ficus-indica*, and also found in citrus fruits, exhibits antioxidant properties that could improve the fatty acid profile of edible products such as milk from ruminants [45]. The significant influence of dietary polyphenols, including tannins and flavonoids that are abundant in *Aloe barbadensis* and *Opuntia ficus-indica*, on milk production and other production parameters has been widely emphasized [4, 15, 17, 46, 47, 68]. Furthermore, dietary condensed tannins have the ability to modify and improve the essential fatty acid composition, including omega-3 and omega-6, while lowering the proportion of unsaturated fatty acids in meat and milk [45, 69].

3.2.3 Wool growth and quality

Although not much information has been published on phytochemical effects on wool production, Beigh et al. [70] observed that the inclusion of herbs rich in terpenoids, flavonoids, and phenolic acids, which are also present in *Aloe barbadensis*, *Hylocereus* spp., and *Portulacaria afra*, improved weight gains that positively affected wool growth and development without any adverse effects on wool quality and yields.

3.2.4 Meat quality and shelf life

Approximately 70% of phytonutrients present in meat from livestock, including sheep, goats, cattle, and their other edible products, mostly originate from the plant-based feedstuffs mixed in diets [16]. Hence, the diet remains one of the paramount factors influencing meat quality, especially its color, flavor, and fatty acid profile, in sheep meat, including both lamb and mutton [4]. Dietary inclusion of plants rich in polyphenolic curcuminoids, such as turmeric (*Curcuma longa*) rhizome extracts, which are also abundant in *Hylocereus* spp. and *Portulacaria afra*, helps prevent lipid peroxidation, brain aging, and neurodegenerative abnormalities in ruminant animals, especially sheep and goats [71]. Uushona et al. [69] also reported that condensed tannins

in *Acacia* leaves (also detected in *Opuntia ficus-indica*, *Echinocereus* spp., and *Aloe barbadensis*) improved the flavor, taste, and shelf life of meat from ruminant livestock when included in diets. This is mainly because of the positive effects of phytochemicals, including tannins, that are abundant in common plant feed ingredients, feed additives, or plant-based diets. Li et al. [72] further emphasized that the antioxidant, antimicrobial, and anti-inflammatory properties of phytochemical-rich plants significantly improve the overall health of meat and its byproducts. According to various authors, dietary supplementation of phytochemicals such as catechins, resveratrol, anthocyanins, and lycopene, present in *Opuntia ficus-indica* and also found in fruits and vegetables, in livestock diets aids in inhibiting lipid peroxidation while improving the stability of muscle meat during storage [73–75]. Other authors also reported that these natural antioxidant agents are essential in the preservation of meat and other edible products by protecting cells, membranes, and vital nutrients from oxidative attacks by free radicals when added in ruminant diets [76].

3.2.5 Inhibiting methane production and emission in sheep

According to Saeed et al. [77], higher concentrations of organo-sulphur compounds, saponins, essential oils, flavonoids, and tannins present in *Aloe barbadensis* assist in reducing methanogenesis by altering the rumen microflora such that they inhibit the growth and reproduction of rumen bacteria species responsible for methane production. Additionally, the supplementation of plants rich in saponins, such as *Aloe barbadensis*, inhibits ammonia nitrogen levels in the rumen during microbial fermentation [10]. Whereas high dietary tannin concentration causes increased antioxidant activity that positively modifies the growth and functioning of rumen microbes, mainly beneficial bacteria. This becomes beneficial in helping to reduce bloating, nitrogen, and methane gas emissions in ruminant species such as sheep [10, 45, 46, 69].

3.3 Limitations of supplementing phytochemical rich-succulent plants in sheep diets

The appropriate dosage of dietary phytonutrients, especially oxalates, phytates, saponins, and tannins found in succulent shrubs such as *Aloe barbadensis* pulps (Table 2) is highly determined by their chemical structures, phytochemical additives, diet, and the physiological status of the animal [63]. According to Guan et al. [12], dietary supplementation of plants with excessive levels of phytochemicals in ruminant diets could become toxic to the host animal. For instance, dietary inclusion of *Aloe barbadensis* and *Atriplex nummularia*, documented to contain high concentrations of oxalates, could result in poisoning or death in ruminant animals [34, 55]. Hence, care needs to be taken to avoid overdosage or inclusion of plant feedstuff or extracts in sheep rations to maintain the overall health of sheep, which may ultimately result in severe complications in humans.

4. Conclusion

Phytochemicals play a huge role in the growth, development, and proper functioning of plants, such as succulent shrubs, that could potentially be added to

sheep diets as supplements. Edible succulent shrubs, such as *Opuntia ficus-indica* and *Aloe barbadensis*, generally contain lower nutrient content but are high in moisture and phytochemicals. The moisture content could be useful in providing additional water for sheep reared in areas constantly experiencing droughts and water shortages, whereas phytonutrients present in succulent plants could aid in enhancing phytochemical concentrations within the muscles, organs, and tissues, which function to provide protection mainly against aging and diseases in sheep. This is because they mainly act as natural antioxidants, preventing the formation of free radicals during lipid peroxidation, thus preserving nutrients while improving the health, quality, stability, and shelf life of sheep meat and other edible products. Hence, there is a need to fully explore the potential utilization of succulent plants as a supplement in sheep diets to improve the health and productivity of various sheep breeds.

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Conflict of Interest

There are no conflicts of interest.

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Azolla Pinnata as a Sustainable Sheep Feed: Nutritional Value, Conservation Methods, and Performance Outcomes

Soumaya Boukrouh

Abstract

Azolla pinnata is a valuable alternative feed resource for sheep, and its nutritive profile and performance are strongly influenced by conservation methods and inclusion levels. Fresh *Azolla*, with low dry matter (7.05%), is rich in minerals, protein (17–29%), and essential amino acids, supporting high palatability and digestibility but limited by perishability. Sun- and shade-drying increase dry matter (91%) and storage potential with minimal protein loss, although sun drying can raise crude fiber and lignin and cause mineral or amino acid reductions. Performance trials revealed that moderate inclusion of fresh or shade-dried *Azolla* (9–12% dry matter intake) improved body weight gain, feed conversion ratio, carcass traits, and economic efficiency. Sun-dried *Azolla* at ≤10% inclusion can be beneficial; however, higher levels often reduce intake, digestibility, and growth, likely due to a decline in palatability or the presence of anti-nutritional factors. Fresh *Azolla* enhances metabolic, reproductive, and immune responses, whereas shade-dried *Azolla* maintains its digestibility and rumen function. Across studies, optimal benefits occurred at low-to-moderate inclusion tailored to the conservation method and feeding duration. The findings of this review highlight the potential of *Azolla* as a low-cost, sustainable supplement for sheep, contingent on processing choices that balance nutrient conservation, storability, and animal performance.

Keywords: *Azolla pinnata*, sheep, nutritive value, digestibility, milk, meat

1. Introduction

Ensuring an optimal nutrient supply and bioavailability is crucial for enhancing livestock productivity, particularly in resource-limited areas [1]. However, in many developing countries, rangeland degradation and the shortage of arable land for fodder production, coupled with urbanization, population growth, and the need to conserve water resources, have intensified feed scarcity, especially during the dry season [2]. Under these challenging conditions, some animal species, such as camels, have become environmentally and economically advantageous [3–5].

Traditional protein-rich feedstuffs, such as soybean meal, are becoming increasingly expensive, especially under climate change conditions, with feed costs accounting for 50–60% of the total expenditure in sheep production [6]. This economic pressure has driven nutritionists and livestock producers to look for non-conventional, locally available, and even marginal feedstuffs that are cost-effective and can partially or totally replace conventional protein sources without compromising animal health or performance [7–14].

Among emerging alternatives, *Azolla pinnata*, a small free-floating aquatic fern, has gained significant attention owing to its exceptional nutritional value and rapid biomass production [15]. Growing in symbiosis with the nitrogen-fixing cyanobacterium *Anabaena azollae* [16], *Azolla* can accumulate 20–35% crude protein and essential amino acids, such as lysine, isoleucine, and arginine, and is rich in minerals (Ca, P, K, Fe, Cu, Mg), vitamins (A, B12, β -carotene), antioxidants, and growth-promoting compounds [17, 18]. Its low lignin content and high digestibility make it an efficient protein supplement for ruminants [19, 20]. Moreover, its adaptability to tropical and subtropical aquatic environments allows year-round cultivation with minimal input.

Previous studies have demonstrated the potential of *Azolla* species as partial substitutes for conventional protein feed in goats [21], cattle [22], poultry [23], pigs [24], and fish [25]. For sheep, only *Azolla pinnata* was tested. Owing to its low production cost, rapid growth, and suitability for integration into smallholder and semi-intensive systems, *Azolla pinnata* could be a promising sustainable feed option. Despite its potential, the nutritive value of *Azolla pinnata* is influenced by conservation methods, which can affect moisture content, nutrient stability, and storability. Understanding these conservation-induced changes is critical for optimizing feeding strategies.

This chapter examines the nutritional characteristics of *Azolla pinnata* under different conservation methods; evaluates its effects on digestibility, rumen fermentation, growth performance, carcass quality, and economic efficiency in sheep; and discusses its role as a sustainable alternative feed resource. This study aims to provide evidence-based guidance for integrating *Azolla pinnata* into sheep feeding systems to balance nutrient conservation, animal performance, and cost-effectiveness.

2. Materials and methods

2.1 Literature search strategy

A comprehensive literature search was conducted to collect published data on the nutritive value, conservation methods, and performance effects of *Azolla pinnata* in sheep diets (**Figure 1**). Peer-reviewed journal articles, conference proceedings, and technical reports published in English up to July 2025 were retrieved from major scientific databases, including Scopus, Web of Science, ScienceDirect, PubMed, and Google Scholar. The search terms included combinations of keywords such as “*Azolla* spp.,” “sheep,” “meat,” “milk,” “digestibility,” “growth performance,” “meat quality,” and “rumen fermentation.” The reference lists of relevant publications were also screened to identify additional studies.



Figure 1.
CABI International, *Azolla pinnata* (mosquito Fern), 2022, 10.1079/cabicompendium.8120.

2.2 Inclusion and exclusion criteria

This study aimed to evaluate the effects of *Azolla* species on sheep performance. Studies were included if they reported experimental feeding trials on sheep digestibility, rumen fermentation parameters, growth performance, carcass traits, or meat quality, provided quantitative data on the chemical composition, mineral, and amino acid content analyses of *Azolla spp.*, specified inclusion levels and trial durations; and used *Azolla* in fresh, sun-dried, or shade-dried forms. Studies were excluded if they lacked a control group, focused exclusively on ruminant or non-ruminant species other than sheep, or lacked sufficient methodological details to determine conservation methods or feeding regimes.

2.3 Data extraction and processing

The following information was extracted from each eligible study: conservation method, sheep breed, trial duration, inclusion level of *Azolla spp.* in the diet (% dry matter intake or g/day), and measured parameters (chemical composition, fiber fractions, mineral profile, amino acid content, and digestibility coefficients). The main results regarding the effects of *Azolla spp.* on rumen fermentation variables, blood biochemical and immune parameters, growth performance, carcass traits, and economic efficiency were also extracted. Chemical composition data were standardized to a dry matter basis where necessary. Mineral content was expressed as either a percentage or parts per million (ppm), and amino acids were expressed as a percentage of total amino acids.

2.4 Data synthesis and comparative analysis

As *Azolla pinnata* was the only species that had been incorporated into sheep diets, its nutritional value was extracted and tabulated separately under different

conservation methods (fresh, sun-dried, and shade-dried). The ranges, means, and standard deviations were calculated when sufficient data were available. Qualitative synthesis was applied when data heterogeneity precluded direct quantitative comparisons. The influence of the conservation method, inclusion rate, and trial duration on the outcomes was examined descriptively to identify trends and optimal usage levels.

3. Results and discussion

3.1 Nutritive value of *Azolla pinnata* according to different conservation methods

The nutritive value of *Azolla pinnata* shows marked variation depending on the conservation method (fresh, sun-dried, or shade-dried), reflecting changes in chemical composition, mainly moisture and mineral levels, fiber fractions, and amino acid profiles (Table 1).

3.1.1 Chemical composition

Fresh *Azolla* is characterized by a very low dry matter (DM) content (7.05%), indicative of its high moisture content, which may limit intake on a DM basis but favors palatability and digestibility. In contrast, sun-dried and shade-dried forms have DM values of approximately 91%, making them more concentrated feed sources that are suitable for storage and transport. According to Prasad et al. [26], the low dry matter content of *Azolla* can be a limitation for its use in fresh form, as a large quantity is required to meet the dry matter needs of livestock. Although *Azolla* has a low percentage of DM content, it may still be used as a supplement in livestock feed. Indeed, its protein content is high and comparable to that of several legume plants [27, 28]. This higher crude protein (CP) content is attributed to *Azolla*'s unique trait of growing in a symbiotic relationship with the endosymbiotic nitrogen-fixing bacterium, the blue-green algae *Anabaena azollae* [29]. Moreover, the CP content was relatively consistent across conservation methods, with fresh (23.46%), sun-dried (23.36%), and shade-dried (25.02%) samples showing only slight variation, suggesting minimal protein loss during drying. However, large variations were observed under the same conservation method. A possible reason for the variability in CP content is the response of *Azolla* strains to environmental conditions such as light intensity, temperature, fertilization management, and water quality. On the other hand, crude fiber (CF) increased upon sun-drying (15.70%) compared to fresh distribution (12.63%), possibly due to concentration effects and structural changes during dehydration, whereas shade-dried material showed intermediate values (13.30%). Ether extract (EE) content remains similar across treatments (~3.75–3.91%), implying that lipid fractions are relatively stable during drying. Ash content, an indicator of mineral load, was highest in fresh *Azolla* (20.29%), slightly lower in shade-dried *Azolla* (19.60%), and lowest in sun-dried *Azolla* (18.59%). Nitrogen-free extract (NFE), reflecting soluble carbohydrate fractions, was somewhat higher in fresh (38.77%) and shade-dried (38.50%) samples than in sun-dried (36.16%) samples, suggesting that sun exposure may induce carbohydrate degradation. Contrary to what was expected, average fiber fractions

[illegible]

References	[18, 30–42]					[17, 21, 43–57]					[58–61]				
	Fresh					Sun dried					Shade dried				
	N	Mean	Min	Max	SD	N	Mean	Min	Max	SD	N	Mean	Min	Max	SD
Copper (Cu)	3	22.18	17.09	34.19	12.12	1	17.15				1	0.86			
Iron (Fe)	5	2737	714.2	490.0	163.6	2	733.19	710.7	755.7	31.88	1	14.04			
Zinc (Zn)	4	79.68	33.14	230.0	84.87	2	82.45	77.30	87.59	7.28	1	1.56			
Manganese (Mn)	5	703.9	205.2	2000	672.3	2	191.15	174.4	207.9	23.65	1	7.96			
Copper (Cu)	2	9.56	3.12	16.00	9.11	1	16.74								
Nickel (Ni)	1	6.51													
Chromium (Ch)	1	8.11													
Cadmium (Cd)	1	0.08													
Molybdenum (Mo)	1	0.65													
Lead (Pb)	1	1.56													
Amino acids															
Alanine	5	5.16	2.32	7.50	3.16										
Arginine	6	7.93	5.79	11.55	2.81	1	5.37								
Aspartate	5	10.14	9.19	11.25	0.88										
Glutamate	5	19.45	12.60	31.30	8.56										
Glycine	5	5.66	5.47	5.79	0.14	1	4.6								
Histidine	4	2.01	1.49	2.59	0.46										
Isoleucine	6	4.80	3.46	6.25	0.98	1	4.35								

References	[18, 30–42]					[17, 21, 43–57]					[58–61]				
	Fresh					Sun dried					Shade dried				
	N	Mean	Min	Max	SD	N	Mean	Min	Max	SD	N	Mean	Min	Max	SD
Leucine	6	8.88	7.81	10.00	3.72	1	7.71								
Lysine	6	5.24	4.28	6.10	0.73	1	4.58								
Methionine	6	2.53	1.14	5.87	1.84	1	1.59								
Phenylalanine	5	5.16	3.87	6.25	1.07	1	4.72								
Serine	5	4.83	4.30	5.63	0.61	1	4.21								
Threonine	6	4.47	3.22	5.63	0.92	1	4.07								
Tyrosine	5	4.50	3.60	5.63	2.57	1	3.18								
Valine	6	5.57	3.77	6.88	1.10	1	5.51								
Tryptophan	3	2.90	1.41	5.48	2.25	1	1.82								
Cysteine	2	0.85	0.09	1.6	1.07	1	0.84								
Proline	3	4.67	4.43	4.9	2.70										

Table 1.
Nutritive value of Azolla pinnata.

(NDF and ADF) tended to be higher in fresh material, with sun-drying reducing them. However, the results could be due to higher variation in the fresh form (35.18–60.11% DM), possibly because environmental growth conditions have a greater impact than conservation methods. Lignin content increased substantially in sun-dried samples (20.41%) compared to fresh samples (9.91%), which could be an artifact of the relative concentration after soluble matter loss.

3.1.2 Mineral composition

Mineral composition varied widely, with notable increases in calcium in sun-dried (1.57%) samples compared to fresh (1.07%) samples, likely due to contamination or accumulation from drying conditions. Phosphorus is slightly higher in sun-dried (0.77%) than in fresh (0.65%) samples. Sodium, magnesium, and micronutrients such as copper, iron, and zinc also show variable retention depending on the drying method, with fresh samples generally richer in Fe (2737 ppm) and Mn (703.9 ppm), while sun-dried and shade-dried forms show considerable reductions, suggesting potential oxidation or leaching losses.

3.1.3 Amino acid profile

Amino acid profiles are best documented for fresh *Azolla*, which contains high levels of glutamate (19.45%), aspartate (10.14%), leucine (8.88%), and arginine (7.93%), thus providing an excellent source of essential amino acids. Sun-dried samples recorded lower amino acid values, but the available data suggest slight reductions, potentially due to heat-induced denaturation. Shade-dried amino acid values are scarcely reported, but given the milder drying conditions, retention is expected to be higher than that of the sun-dried forms. Overall, fresh *Azolla* provides superior mineral and amino acid availability, but is limited by high moisture content and perishability. Sun-drying increases storability and nutrient density per unit weight but may cause some degradation of sensitive components, particularly minerals and certain amino acids, and can unfavorably alter fiber composition. Shade drying appears to offer a compromise by conserving much of the protein and carbohydrate fractions with less mineral loss, although the data are limited. For sheep diets, the choice of conservation method depends on the production system; fresh *Azolla* is suitable for immediate feeding near production sites, whereas dried forms, especially shade-dried forms, offer logistical advantages for storage, transport, and incorporation into formulated feeds without significant nutrient loss.

3.2 Impact of *Azolla pinnata* on digestibility and rumen fermentation

The use of *Azolla* as a feed supplement in sheep diets has been investigated using various conservation methods, incorporation rates, and experimental durations, revealing that its effect on animal performance and metabolism is influenced by both the form and level of inclusion. The conservation method plays a key role in determining not only nutritional effectiveness, but also the duration of the trial. For example, sun-dried *Azolla* showed both positive and negative outcomes depending on the trial duration (**Table 2**). In male Barki sheep, it improved DM and CP digestibility, increased digestible crude protein (DCP) and total digestible nutrients (TDN), and enhanced rumen fermentation characteristics, such as pH, volatile fatty

Country	Animal	Fresh/ dry	Trial dur. (days)	Incl. levels	Main findings	Rec. levels	Ref.
Egypt	Male Barki sheep	Sun dried	49	Azolla vs. clo- ver hay	Azolla increased digestion coefficients for dry matter (DM) and crude protein (CP), but reduced nitrogen-free extract (NFE) compared to clover hay, with no significant difference in crude fiber (CF). Azolla increased the nutritive values (DCP and TDN%) and N balance compared to clover hay. Azolla significantly improved rumen characteristics (pH, VFA, and NH ₃ -N) after 6 h of feeding.	-	Nayel, et al., [44]
India	Nellore sheep	-	-	0 and 30%	Azolla increased in vitro dry matter digestibility (DMD), in sacco DM disappearance, and CP degradability (%), protein degradation (PD), and energy degradation (ED) over the control diet. Azolla improves blood biochemical profiles, such as serum albumin levels. No significant differences were reported in blood total protein, glucose, total cholesterol, blood urea nitrogen (BUN), creatinine, and hematological values, including hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), and packed cell volume (PCV).	30%	Rao and Reddy, [67]
Egypt	Ossimi rams	Shadow dried	26	0, 2, and 4% DM	2 and 4% DM Azolla extract significantly increased DM digestibility and nitrogen balance by 7.64% and 14.96% above the control group, respectively. They also increased glucose concentration, Immunoglobulin A (IgA), and Immunoglobulin G (IgG). 4% Azolla led to the highest CP, CF, and NFE digestibility, TDN, Digestive crude protein (DCP %), VFA, and NH ₃ -N in the rumen 2 h post-feeding. The 4% DM Azolla group had the highest values of serum total protein and albumin. It also increased Interleukin-2. No significant differences were reported in liver and kidney functions, as well as hematological parameters.	4%	Kumar, et al., [64]
Egypt	Crossbred (Osimi X Fielandi)	Sun- dried	120	0,10, and 20%	10 and 20% Azolla decreased the digestibility of DM, OM, CP, and NFE, as well as the feeding value in terms of TDN and DCP, compared to Co group.	NR	Abou El-Fadel, Hassanein, and El-Sanafawy [68].

Country	Animal	Fresh/ dry	Trial dur. (days)	Incl. levels	Main findings	Rec. levels	Ref.
Iraq	Iraqi Awassi lambs	-	45	0, 4, 8, 12%	4 and 12% Azolla increased total weight gain, but no significant differences were observed in the amount of total feed intake.	12%	Abbas and Mohanned, [69]
					The 12% Azolla treatment showed the lowest FCR. No significant effects were reported for the digestibility coefficients of dry matter, organic matter, and crude protein; however, crude fiber and ether extract were significantly different, with higher values observed for the 12% inclusion rate.		
Iraq	Awassi lambs	Air dried	75	0,3, and 5%	Azolla had no significant effect on rumen pH or total volatile fatty acids (TVFAs). However, there was a significant increase in N-NH ₃ concentration with increasing Azolla levels in the diet. 3 and 5% Azolla significantly decreased the pH value of rumen liquid from lambs at sample times of 2 and 4h after feeding. Furthermore, lambs fed 5% Azolla showed a significant increase in TVFAs concentration after 4h and in N-NH ₃ concentration at both 2 and 4h post-feeding.	5%	Mohammad, et al., [65]
					Treatment with 10 and 20% Azolla tended to reduce plasma urea and creatinine concentrations.		
Iran	Fat-tail uncastrated male Moghani lambs	Sun dried	90	0, 10, and 20%		NR	Vahedi, et al., [63]
India	Bucks	Fresh	120	100 g/ day	Supplementation of Azolla improved reaction time, progressive motility, semen volume, percentage of live, dead, and abnormal spermatozoa, as well as blood characteristics and rumen metabolites.	100 g/day	Kumar, et al., [66]
Egypt	Lamb	Sun- dried	120	10% and 20% CC	10 and 20% CC decreased DMD, NFED, and feeding value as TDN. 20% CC increased ruminal pH and NH ₃ -N. 10% CC showed better economic feed efficiency than 20% CC.	NR	El-Fadel et al., [62]
NR, not recommended; CC, concentrate; dur., duration; Incl., inclusion; rec., recommended; DM: dry matter.							

Table 2.
Impact on digestibility and ruminal fermentation.

acids (VFAs), and ammonia nitrogen ($\text{NH}_3\text{-N}$), compared to clover hay [44]. Conversely, in long-term trials (4 months) with crossbred Egyptian sheep and lambs, high inclusion levels (10–20%) of sun-dried *Azolla* decreased DM, organic matter (OM), CP, and NFE digestibility, as well as feeding value [62], suggesting that prolonged feeding at high rates may impair nutrient utilization. However, in Iranian Moghani lambs, the same conservation method at 10–20% tended to reduce plasma urea and creatinine levels [63], indicating improved nitrogen metabolism and possible kidney function benefits. Shadow drying has also been effective, as observed in Ossimi rams supplemented with 2–4% *Azolla* extract, where DM digestibility, nitrogen balance, serum proteins, immunoglobulins (IgA, IgG), and rumen fermentation parameters improved significantly without negative effects on liver or kidney function [64], underscoring its safety when fed within optimal limits. Air-dried *Azolla* in Iraqi Awassi lambs had no overall effect on rumen pH or total VFAs; however, time-specific sampling revealed reductions in pH and increases in VFAs and $\text{NH}_3\text{-N}$ shortly after feeding, indicating rapid rumen fermentation [65]. Fresh *Azolla* appears to be particularly beneficial in conserving bioactive compounds, with supplementation at 100 g/day in bucks enhancing semen quality, motility, sperm viability, and rumen metabolite levels [66]. The effect of *Azolla* was closely linked to the incorporation rate. Low inclusion levels ($\leq 5\%$ DM) generally produced positive or neutral effects on digestibility, nitrogen balance, and rumen fermentation, as observed with 2–4% DM in Ossimi rams, which yielded consistent improvements. Moderate inclusion (10–12% DM) produced mixed responses: in Iraqi Awassi lambs, 12% improved total weight gain and feed conversion ratio (FCR) without affecting DM, OM, or CP digestibility, although fiber digestibility increased; however, in Egyptian crossbred sheep, the same range decreased overall digestibility and feeding value. High inclusion levels ($>20\%$) were rarely tested but were generally associated with reduced digestibility and energy value, potentially due to excess fiber, anti-nutritional compounds, or reduced palatability. Overall, evidence suggests that shadow-dried or fresh *Azolla*, incorporated at 2–12% DM depending on the production goal and feeding duration, can enhance digestion, metabolism, and even reproductive or immune functions in small ruminants, whereas higher inclusion levels and prolonged feeding may require caution to avoid reductions in nutrient utilization.

3.3 Impact of *Azolla pinnata* on growth performance and meat quality

3.3.1 Impact on growth performance

These studies revealed considerable variation in the effects of *Azolla* supplementation in sheep diets, with outcomes influenced by the conservation method, inclusion rate, and duration of feeding trials (**Table 3**). Fresh *Azolla*, as reported by [69–71], generally produces positive growth performance at relatively low inclusion levels (around 3–12%), enhancing final body weight (FBW), body weight gain (BWG), average daily gain (ADG), and FCR, while also reducing feeding costs. Higher fresh *Azolla* inclusion (20% DMI) maintained benefits in FBW [72, 73] and body condition score (BCS) [47], but showed less consistent improvement in ADG, suggesting that palatability or nutrient imbalance might limit gains at elevated levels. Shade-dried *Azolla* has been used in previous studies [73, 74], which also supported performance improvements at 10–20% inclusion, with increased ADG

Country	Animal	Fresh/ dry	Trial dur. (days)	Incl. levels	Main findings	Rec. level	Ref.
India	Ram lamb	Fresh	120	10% and 20% DMI	10 DMI of Azolla increased final body weight (FBW), BW gain, average daily gain (ADG), and final body condition score (BCS), while 20% DMI increased only FBW and BCS.	20% DMI	Harani, et al., [72]
India	Sheep	Shade dried	120	10% and 20% DMI	10 and 20% DMI increased FBW, ADG, and cumulative WG.	20% DMI	Reddy et al., [73]
India	Nellore sheep	-	-	0 and 30%	Azolla increased total BW gain and ADG compared to the control in both systems but reduced the feed conversion ratio (FCR). Azolla reduced the cost of feed/kg WG by ~ 50% in experimental diets compared to the control diet.	30%	Rao and Reddy [67]
India	Lamb	Fresh	90	9.5 and 9.7%	9.5 and 9.7% Azolla increased ADG, improved FCR, and reduced the cost per kilogram of weight gain.	9.7%	Krishna et al., [70]
India	Sheep	Sun dried	90	6, 12, 18, and 24%	24% Azolla decreased dry matter intake (DMI). 12%, 18%, and 24% Azolla decreased FBW, BW gain, ADG, and increased FCR and cost of feeding/kg.	0%	Ahmed, et al., [19]
India	Sheep	Shade dried	90	10% CC	No effect was reported on DMI, ADG, or feed efficiency.	10% CC	Sankar, et al., [20]
India	Deccani ram lambs	Shade dried	120	0, 10, and 20%	10 and 20% Azolla significantly increased the cumulative WG and ADG compared to Co diet.	20%	Singh, et al., [74]
Egypt	Crossbred (Osimi X Fielandi)	Sun dried	120	0, 10, and 20%	20% Azolla recorded the lowest FBW, total BW gain, and ADG compared to Co. However, 10% Azolla recorded the best economic feed efficiency (%) followed by the 20% Azolla group.	10%	Abou El-Fadel, Hassanein, and El-Sanafawy [68]
Iraq	Iraqi Awassi lambs	-	45	0, 4, 8, 12%	12% Azolla registered the largest increases in FBW and ADG compared to other treatments and the Co diet.	12%	Abbas and Mohammed, [69]

Country	Animal	Fresh/ dry	Trial dur. (days)	Incl. levels	Main findings	Rec. level	Ref.
Iran	Fat-tail uncastrated male Moghani lambs	Sun dried	90	0, 10, and 20%	Treatments with 10% and 20% Azolla had no impact on meat quality attributes. Body weight (BW) was not affected by dietary supplementation of Azolla, but the inclusion of Azolla in the feedlot ration improved the FCR. 10% Azolla increased hot and cold carcass weights (HCW and CCW), but no significant impact was reported for other carcass traits, the chemical composition of the longissimus muscle, or meat sensory properties. Treatment with 20% Azolla increased odor intensity scores compared to the control group.	20%	Vahedi, et al., [63]
Iraq	Awassi lambs	Shade dried	90	0, 3, and 5%	3 and 5% Azolla improved carcass characteristics for the traits (EBW, HCW, and CCW) and slaughtering residues. The 5% Azolla diet increased the meat moisture content compared to the 3% and Co diets. The 3% Azolla showed the highest protein content and percentage of weight in the main pieces (thigh, shoulder), ribs, and carcass compared to the other treatments. There were no significant differences in each of the secondary cuts of the carcass, as well as the neck and buttocks, the area of the ocular muscle, the thickness of the fat layer, slaughter residues, the physical separation of the proportions of the ribs, and the chemical analysis results (ratios of fat, ash, and carbohydrates).	5%	Al Baldawy and Mohammad, [71]

NR: not recommended; CC: concentrate; dur.: duration; Incl.: inclusion; rec.: recommended; DM: dry matter.

Table 3.
Research concerning the use of Azolla spp. In ruminants.

and cumulative weight gain, indicating that nutrient conservation during shade drying may retain much of the feed value. However, Sankar et al. [20] reported no significant effect of shade-dried *Azolla pinnata* on DMI, ADG, or feed efficiency with only a 10% CC inclusion level. In contrast, sun-dried *Azolla pinnata* outcomes were more variable. Ahmed et al. [19] observed that high inclusion rates (12–24%) decreased DMI, FBW, and ADG, while increasing FCR and feeding costs, highlighting a potential negative effect of excessive inclusion, possibly due to reduced palatability or anti-nutritional factors intensified by sun-drying. El-Fadel et al. [62] found that 10% sun-dried Azolla offered the best economic feed efficiency, whereas 20% sun-dried Azolla reduced growth performance (FBW, total BW gain, and ADG), further supporting a moderate inclusion approach.

3.3.2 Impact on meat quality

Meat quality and carcass trait data [63, 71] indicate that moderate inclusion (3–10%) can enhance carcass weight and specific cuts without adverse effects on most sensory attributes, although higher levels (20%) may alter meat characteristics, such as odor intensity or moisture content. Alternative feed resources are used because of their economic benefits. Several studies have reported significant reductions in feed cost per kilogram of weight gain, sometimes up to 50% [62, 67]. However, when used as sun-dried and at the maximum rate (24%), there was an increase in feeding costs, underscoring Azolla's potential as a cost-effective supplement when used judiciously. Optimal inclusion rates varied across studies, but a general trend emerged: fresh or shade-dried Azolla at 3–20% of DMI and sun-dried Azolla at $\leq 10\%$ tended to balance growth performance, carcass quality, and feed efficiency while minimizing potential negative impacts. Beyond growth and carcass traits, some trials reported no significant changes in certain chemical composition parameters of the meat, suggesting that Azolla can be integrated without compromising product quality, provided that inclusion remains within optimal thresholds. However, excessive incorporation, particularly in sun-dried Azolla, may impair intake and performance, indicating the importance of adjusting rates based on conservation methods and animal responses. Overall, the data suggest that Azolla is a promising, low-cost, and nutritionally beneficial supplement for sheep diets, with success depending on the careful selection of conservation techniques and inclusion levels to maximize performance while avoiding diminishing returns. As a future perspective, Azolla can be used in the diets of other species, such as camels [75, 76].

4. Conclusion

Azolla pinnata demonstrates considerable potential as a sustainable feed supplement for sheep, offering a rich profile of proteins, minerals, and essential amino acids. However, its nutritive value is strongly influenced by conservation methods, with fresh Azolla retaining higher moisture and certain heat-sensitive nutrients, whereas sun- and shade-drying reduce moisture but may alter protein quality and mineral availability. Integrating Azolla into sheep diets, whether fresh or conserved, can improve digestion coefficients, nutrient utilization, and overall animal performance, particularly when used at optimal inclusion rates. These benefits not only support animal health and productivity but also offer an

environmentally friendly alternative to conventional feeds, reducing feed costs and pressure on traditional forage resources. Future research should focus on refining conservation techniques to minimize nutrient losses and evaluating the long-term effects on growth, reproduction, and meat or milk quality, ensuring that the full potential of *Azolla* is harnessed in small ruminant production systems.

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Section 3

Reproduction

Chapter 5

Enhancers of Sperm Motility and Viability in Sheep

Adel R. Moawad and Abdallah M. Shahat

Abstract

Sheep possess significant potential for genetic enhancement in terms of meat, milk, and wool production. The total population of sheep in the United States was determined to be 5.05 million. The rise in sheep meat consumption, particularly in the United States, may be partially addressed by enhancing reproductive efficiency. Artificial insemination (AI) has a potential impact on sheep reproduction by improving genetic gain. Nonetheless, reduced fertility rates following AI in sheep coincide with their semen quality, which is influenced by various factors such as breed, method of collection, male nutrition, and freezing methods. Ram spermatozoa exhibit significant vulnerability to oxidative damage, mostly because of the elevated levels of polyunsaturated fatty acids (PUFAs) in their plasma membranes and the excessive generation of reactive oxygen species. Herein, we will highlight some factors that are known to impact ram semen quality. Additionally, we will address various strategies to improve the quality of ram semen and, subsequently, its fertility after AI.

Keywords: sheep, semen quality, artificial insemination (AI), oxidative stress, antioxidants, fertility

1. Introduction

In several countries, sheep production is considered an essential component of animal husbandry, but it varies based on market demand [1]. Sheep have long been raised for meat, milk, and wool production due to their remarkable environmental adaptation [2]. Globally, the total population of sheep and goats is 1.32 billion and 1.14 billion, respectively [3]. Sheep and goats are mostly found in Asia (45.8% and 50.7%, respectively), followed by Africa (31.7% and 44.2%, respectively), Europe (9.1% and 1.3%, respectively), the Americas (6.2% and 3.4%, respectively), and Oceania (7.2% and 0.4%, respectively) [3]. According to estimates, there will be 2.5 million goats and 5.05 million sheep in the United States as of 2025 [4]. Before 2011, the consumption of lamb meat and mutton was steady or dropping. However, its trajectory shifted significantly after 2011, with constant increases [5]. The consumption of lamb meat and mutton in the United States increased from 295 million pounds in 2011 to 484 million pounds in 2022 [5]. However, domestic production has not kept pace with this rising demand, creating a substantial gap between consumption and production. This widening deficit underscores the urgent need to improve sheep productivity to enhance national

production capacity and reduce reliance on imported lamb and mutton. This gap can be narrowed by boosting sheep production, which can be achieved by improving the reproductive efficiency of both ewes and rams. Animals' ability to reproduce is one of their most crucial characteristics, as it affects the flock's total profitability. One of the pivotal characteristics that impacts the ovine industry's economic competence and productivity is their fertility, which is influenced by different factors, including breeds, male and female reproductive health, season, age, and management.

Livestock productivity can be enhanced by disseminating superior genotypes and reducing generation intervals through the utilization of assisted reproductive technologies (ARTs). This includes artificial insemination (AI), estrus synchronization, superovulation, non-surgical embryo collection, embryo transfer, cryopreservation of embryos, oocyte pick-up from live animals, *in vitro* embryo production (IVEP), and cloning. Artificial insemination is the first technique that was implemented to enhance the genetics of farm animals, allowing the rapid spread of favorable male traits with high productivity. However, the applications of ARTs, specifically AI, are still not well employed in small ruminants compared to bovines [6, 7]. Sheep AI is limited by several obstacles, which include the anatomical features of the ewe's cervix and the quality of ram spermatozoa [8]. Sperm preservation techniques, such as cooling and cryopreservation, must also be considered, as they are intricately associated with the type of sperm utilized in AI. Notwithstanding significant advancements in the types of semen extenders [9, 10], alterations in cooling and freezing protocols [10, 11], and variations in spermatozoa concentration during storage [10], the procedure can still cause substantial ultrastructural, biochemical, and thermal damage [12–14], resulting in diminished or inconsistent post-thaw semen quality and fertility rates. Sperm quality is one of the intrinsic factors that impact the success rates of AI. In this chapter, we will highlight some factors that affect ram semen quality and various strategies that can be employed to improve the quality of ram semen and, subsequently, its fertility after AI.

2. Materials and methods

We achieved our objective using a narrative review, which was carried out from July to December 2025. The databases searched were PubMed, Google Search, and Google Scholar. We used terms including “semen quality in sheep/rams,” “artificial insemination in sheep,” “antioxidants in rams' semen,” “dietary antioxidants in rams,” “oxidative stress,” and “rams' fertility.” A total of 138 articles were retrieved from the search and included in our work. These studies were conducted using different breeds of sheep, in which semen was collected by different methods and during different seasons and climatic conditions.

3. Factors affecting semen quality in sheep

3.1 Internal factors related to the animal

3.1.1 Age

Research indicates that age has a substantial and relatively uniform impact on ram semen quality, although the link is complex [15–18]. Semen volume, sperm

concentration, and total sperm output per ejaculate generally augment with age, reaching their peak around three years, commonly ascribed to an increase in scrotal size [16, 17, 19–22]. While several studies report improved sperm motility and membrane integrity in mature and even older rams (≥ 8 years) [15, 21–23], other findings show a decline in motility after peak maturity, suggesting breed-specific variations [20, 24]. Findings on sperm morphology are inconsistent, with reports of reduced, increased, or unchanged abnormality rates with the ram's age [16, 18, 20, 24, 25]. Limited data on chromatin integrity suggest no significant age-related differences in DNA compaction or lipid peroxidation [21, 25]. Semen quality seems to peak at three years of age, followed by a variable drop; yet older rams can retain similar or superior sperm viability compared to younger rams [22].

3.1.2 Breed

The effect of sheep breed on semen quality is evident, with considerable variation observed in scrotal circumference, sperm concentration, and ejaculate volume among different genetic lines. Scrotal circumference, a key indicator of testicular development and spermatogenic potential, ranges from approximately 28 cm in young Ile-de-France and Finn rams [26, 27] to 35 cm or greater in Charmoise, Karakul, and Katahdin breeds [19, 28, 29]. Similarly, sperm concentration varies widely across breeds, from relatively lower values in Debouillet and Creole rams ($1.17\text{--}2.79 \times 10^9/\text{mL}$) [30] to higher concentrations exceeding $4.0 \times 10^9/\text{mL}$ in Pelibuey, Awassi, and Corriedale breeds [19, 28, 29]. Semen volume also differs, with reports ranging from 0.6 mL in Katahdin [31] to 2.81 mL in Hampshire rams [30]. These marked breed-specific differences in seminal traits underscore the significant genetic influence on ram reproductive potential and highlight the importance of considering breed as a key factor in semen evaluation and breeding program design.

3.1.3 Parasitic infection

Parasitic infection in sheep is a prevalent condition that has been documented to negatively influence their health, productivity, and welfare [32, 33]. Due to the extensive anthelmintic resistance in many regions of the world, trichostrongylid gastrointestinal infections are currently one of the biggest challenges in sheep health management [32, 34]. Previous research claimed that parasitic diseases can influence the scrotal circumference of rams, which makes parasitic control critical for their reproductive health [35]. It is known that scrotal circumference is linked to a ram's reproductive performance [36]. However, Gaglio et al. [37] found no significant effect of gastrointestinal trichostrongylid infection on semen parameters in rams. Regarding the effect of parasitic infection on ram semen quality, it has been shown that Berberine rams infected with *Haemonchus contortus* experienced harmful effects on their health, semen quality, and reproductive endocrinology [37]. Infected rams, which received 30,000 third-stage larvae orally, exhibited marked declines in key hematological parameters, including hematocrit, red blood cell count, and hemoglobin, starting 21 days post-infection, alongside reduced albumin and total protein levels [37]. Semen quality was also compromised, with significantly lower sperm concentrations on days 69 and 76 and a higher sperm mortality rate on day 46 post-infection compared to the controls [37]. Furthermore, infected rams showed lower plasma testosterone levels and reduced testosterone pulse frequency, although

luteinizing hormone (LH) concentrations remained unaffected [37]. These findings demonstrate that haemonchosis in rams not only impairs general health but also directly undermines reproductive performance through compromised spermatogenesis and hormonal disruption [35]. Moreover, Rhodes [38] demonstrated that extensive infection with chorioptic mange on the scrotum of the rams resulted in a reduction in their semen quality due to seminal degeneration. This was explained based on the long-term elevation in intra-scrotal temperature induced by inflammation [38]. In the same study, the authors found that semen quality was restored to normal values after the skin lesions were treated [38]. These findings assure that parasitic infestations can directly impair testicular function, testosterone production, and consequently semen quality in rams, leading to a decline in their fertility and a consequent reduction in conception rates within the flock.

3.2 External factors related to the environment

3.2.1 Season/photoperiod

In sheep reared in the northern hemisphere, environmental factors, including the photoperiod or the quantity of light, significantly influence reproductive regulation. The concentration of melatonin, a hormone that is secreted from the pineal gland and responsible for reproductive regulation and seasonal breeding in sheep, rises in fall and winter when there is less natural light and reduces in summer and spring when the natural daylight is longer [39]. Decreasing the natural daylight enhances the sensitivity of the pineal gland to secrete high levels of melatonin, which positively impacts the hypothalamic–pituitary–gonadal (HPG) axis, leading to an increase in testosterone secretion [28, 39]. It has been shown that a high level of testosterone is associated with elevation in male sexual behavior and production of viable spermatozoa [39]. Research revealed that the period between late August and February in the northern hemisphere (breeding season) is marked by the recommencement of testes activity and a rise in gonadotropin-releasing hormone (GnRH), LH, follicle-stimulating hormone (FSH), and testosterone production [39–41]. On the contrary, adult rams' and ram lambs' fertility showed a decline during spring and summer months (the nonbreeding season) due to the reduction in sperm production and quality [42]. The impact of the breeding season on the quality of ram semen has also been demonstrated in numerous studies. For instance, Moghaddam et al. [43] noticed that semen samples obtained during the nonbreeding season (spring and summer) had considerably lower volume, sperm concentrations, motility, and the percentage of live spermatozoa than those obtained during the breeding season (fall and winter). However, samples collected during the nonbreeding season showed an increase in sperm abnormalities [43]. Additionally, we found that testicular and epididymal morphometrics, including weights and lengths, epididymal sperm motility, viability, and membrane integrity, were considerably higher during the breeding season (September to November) than the nonbreeding season (July to August) in testicular samples retrieved from slaughtered sheep [44]. We also noticed that the post-thaw sperm motility, viability, acrosome integrity, and mitochondrial activity increased in the samples collected during the breeding versus nonbreeding seasons [44]. Our findings and others highlight the imperative roles played by the breeding season on the quality and functions of ram spermatozoa [43, 44].

3.2.2 Nutrition and body condition score

Nutrition significantly influences reproductive processes and, therefore, is a critical consideration during both the breeding and nonbreeding seasons in sheep. The plan of nutrition is known to impact the body condition score (BCS), which is associated with ram fertility [45]. It has been recommended that six to eight weeks before the beginning of the breeding season, rams should be evaluated for their body condition status [46, 47]. Rams and ram lambs should have a minimum BCS of 3 (on a scale of 1 to 5, where 1 denotes emaciation and 5 indicates obesity) before the breeding season begins [47]. In the meantime, it has been shown that rams' testicular growth and sperm production are influenced by their nutrition plan [48, 49]. Moreover, a strong correlation has been documented between testicular size, sperm quality, and rams' reproductive activity [50]. For maturing ram lambs intended for reproduction, forage as a sole source of nutrition often fails to provide adequate nourishment [51]. The impact of BCS on the reproductive capacity of Malpura rams was studied over a period of 45 days, showing that rams with moderate BCS (3.0–3.5) had higher semen volume, mass and progressive motility, and plasma testosterone levels than those with 2.5 or 4.0 BCS, demonstrating greater reproductive performance in those rams with moderate BCS [47]. Furthermore, the effects of diet levels (high, maintenance, and low) on sperm quality and scrotal circumference of mature Merino rams fed for 65 days have been previously reported [50]. The authors found that scrotal circumference, semen volume, sperm cell concentrations, and sperm velocity parameters were significantly lower in the low-diet group compared to the high-diet one. However, the values of the DNA fragmentation index were higher in the sperm collected from the rams fed a low diet than in those collected from high-diet rams [50]. These observations assure that choosing a strategic nutritional plan is essential to support testicular development, enhance spermatogenic efficiency, and maximize seminal quality and fertility in rams, particularly in preparation for the breeding season.

3.2.3 Heat stress

Heat stress (HS) has a significant impact on animals' ability to reproduce as long as global warming persists, particularly for animals raised in intensive systems like sheep. Low semen quality in rams exposed to HS has been attributed to changes in the neuroendocrine, physiological, and metabolic milieu [52, 53]. Whether caused by scrotal insulation or by exposing the entire body to high temperatures, the harmful effects of HS on testicular health and sperm functions are widely recognized and frequently apparent within 10 to 21 days following exposure to HS [54–56]. For example, earlier research revealed that rams exposed to HS had lower semen volume, sperm concentrations, motility, and viability than non-HS rams [55, 56]. On the contrary, the percentages of sperm with abnormal morphology, degraded plasma membranes, and DNA fragmentation increased in the semen collected from the HS group [55, 56]. Another study in Brazil showed that exposure of Santa Inês hairy rams to HS by scrotal insulation for seven days reduced sperm motility and concentrations directly and at day 8 after removal of the insulation, respectively [57]. However, these values returned to normal profiles, similar to those before treatment, at 79 and 90 days after removal of insulation, respectively [58]. In the same study, the percentages of secondary sperm abnormalities continued to increase up to 24.4%

until the end of the experiment, 118 days after HS exposure [57]. The authors summarized that a temporary interruption of spermatogenesis could be induced by HS in the ram, and sperm motility and secondary abnormalities are the most vulnerable parameters to deterioration [57]. Due to impaired spermatogenesis and increased oxidative stress induced by the excessive production of reactive oxygen species (ROS) in the testicles, HS had a detrimental effect on the quality of ram semen [59, 60]. As a result, important seminal parameters like motility, membrane integrity, and DNA integrity consistently decrease, jeopardizing male reproductive effectiveness in warmer regions.

3.2.4 Semen handling

Proper semen handling practices, including the collection method, frequency of collection, semen cooling, and freezing, are all essential for preserving seminal viability and subsequent rams' fertility. The two methods used for collecting semen in small ruminants are electroejaculation (EE) and artificial vagina (AV). Although AV is believed to be the most effective way to collect semen from rams [58], the males must first be trained to mount a female or a dummy [58, 61]. Sperm concentrations, motility, and viability were observed to be considerably greater in AV-collected semen compared to EE-collected semen in sheep and goats [61–63]. However, there was no distinctive variation in sperm abnormalities between AV- and EE-obtained semen samples in rams [61]. Surprisingly, spermatozoa collected by EE were found to be more resistant to cryodamage than those obtained by AV [62]. Moreover, sperm quality is not only influenced by the method of collection but also by the frequency of semen collection. Research revealed that rams with high collection frequencies (every two days) had significantly lower sperm motility, concentration, and viability, as well as more sperm DNA fragmentation in the second ejaculate, than rams with medium (every four days) and low (every eight days) collection frequencies [64]. Importantly, these deleterious effects were also noted in post-thaw samples, with the high-frequency group showing lower post-thawed sperm motility [64]. Another study noticed that high-frequency collection can increase apoptotic-like changes in the sperm and reduce antioxidant enzymatic activity, leading to oxidative damage [65]. Contrary to these notions, another study claimed that the frequency of semen collection did not have an impact on the quality of fresh semen if the semen was used directly after collection for AI or for *in vitro* fertilization (IVF) purposes [65, 66]. For sheep reproductive programs, efficient semen handling is essential, especially considering the difficulties associated with each stage of AI.

The viability and quality of semen can be greatly impacted by different factors, including temperature changes, mechanical agitation during transportation, and exposure to ROS prior to AI [67]. These factors should be carefully addressed to achieve high fertility rates after AI in sheep [68]. A schematic diagram showing the various factors that can affect semen quality in rams is presented in **Figure 1**.

4. Oxidative stress and semen quality

Sheep AI relies on chilled and frozen/thawed semen [69–71]. Nevertheless, cooling and cryopreservation processes result in a decline in sperm motility,

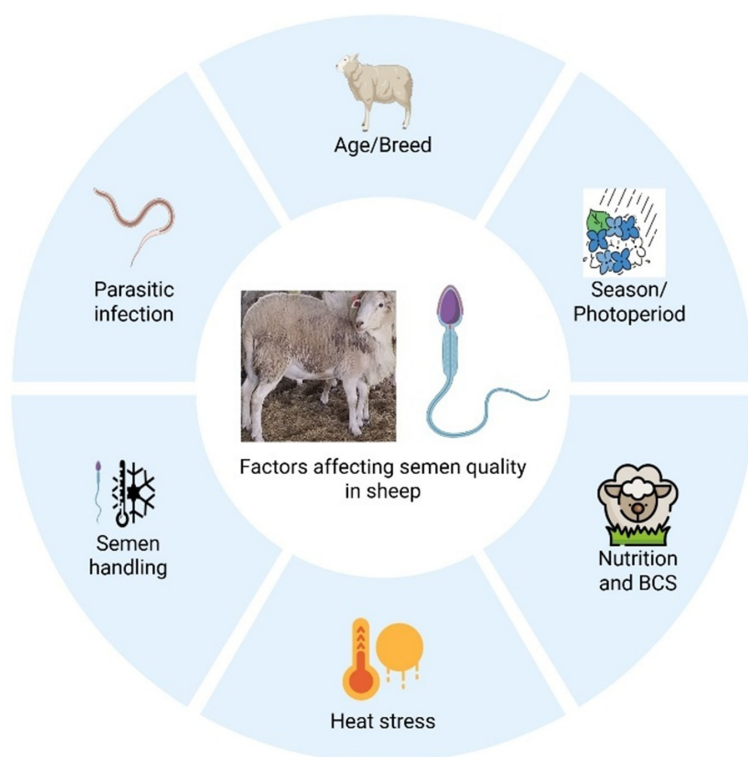


Figure 1.
 Schematic diagram showing factors affecting semen quality in sheep; BCS: body condition score (Source: Developed by the authors through biorender.com).

viability, and membrane integrity [58, 69–71]. One of the main physiological factors causing this susceptibility is the high quantity of polyunsaturated fatty acids (PUFAs) in ram spermatozoa's plasma membrane, making them more vulnerable to cold shock and lipid peroxidation in the presence of ROS [71–74]. This oxidative damage during preservation remains a significant barrier to maintaining post-thaw sperm functions and achieving high fertility rates after AI.

ROS generation in ram spermatozoa is exacerbated by many physiological and handling stresses, including semen chilling and cryopreservation [71, 75, 76], handling during AI [77], exposure to environmental HS [53, 75, 76], and the natural metabolic changes that take place outside the breeding season [78]. Collectively, these factors elevate oxidative stress, leading to a deterioration in sperm functions and subsequent impairment in male fertility [79].

Oxidative stress is described as a breakdown in the regulation of redox signaling caused either by the overproduction of ROS or the exhaustion of regulating antioxidant systems [80]. Maintaining a healthy equilibrium of ROS generation and recycling within the sperm cells is crucial for appropriate physiological activity. Any imbalance can affect sperm functions by causing oxidative stress, which leads to increased lipid peroxidation, resulting in motility loss over prolonged storage [81, 82]. This stress poses a significant challenge to sperm quality, as elevated ROS levels induced by semen handling, preservation, environmental factors, and metabolic

changes can damage sperm at the molecular level. Such oxidative damage impairs key aspects of sperm functions, including motility, membrane integrity, and DNA integrity. For example, ram sperm are known to produce substantial hydrogen peroxide during processing, which directly contributes to post-thaw decline in sperm motility [83, 84]. Therefore, managing oxidative stress through antioxidant supplementation is necessary to mitigate these adverse effects and preserve sperm fertility potential.

5. Enhancement of ram semen quality and subsequent fertility

Mammalian sperm have an antioxidant defense system that includes enzymatic components such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione reductase (GR), as well as non-enzymatic antioxidants like methionine, ascorbic acid, and α -tocopherol [85–87]. The biosynthetic capacity of sperm for endogenous antioxidants is restricted, and dilution for cooling and cryopreservation purposes can diminish the endogenous antioxidant defense systems in the semen, leading to oxidative damage [88]. Consequently, antioxidant supplementation can protect spermatozoa, replenish the redox balance, and enhance fertility outcomes. This applies whether it is given *in vivo* (directly to the animal via feed, injection, or other routes) or *in vitro* (added directly into the semen extenders during cooling and cryopreservation). This demonstrates how exogenous antioxidants can be therapeutically beneficial when endogenous antioxidant defenses are inadequate to mitigate the detrimental effects of ROS [71, 87].

5.1 *In vivo* antioxidants

Antioxidants delivered directly to the animal (*in vivo*) have become an essential strategy for protecting ram sperm against oxidative stress [89]. Administering antioxidants systemically in the form of oral supplementation, injection, or slow-release ear implants can enhance the ram's endogenous antioxidant defense mechanisms by boosting enzymatic activity and scavenging free radicals before they affect spermatogenesis or epididymal sperm maturation [53, 89].

5.1.1 Vitamin E and selenium

Vitamin E is a key fat-soluble antioxidant present in cell membranes. It reduces oxidative damage by stopping the peroxidation chain reaction and limiting the production of ROS. Additionally, it protects cell membranes from excessive ROS by neutralizing free radicals [90]. Besides, selenium (Se) is another molecule that plays a critical role in several reproductive processes, such as testicular growth, spermatogenesis, and testosterone metabolism [91]. Selenium plays a significant role in sperm capsule selenoproteins, resulting in an increase in GPx activity and shielding the sperm membrane from ROS peroxidation [92]. It has been shown that the inclusion of vitamin E in rams' diets can improve their reproductive capacity (see **Table 1**). According to a previous study, supplementing the sheep diet with 200 IU/male per day of vitamin E for a period of 12 months could enhance semen quantity and quality in comparison to the control groups (without vitamin

Antioxidants	Supplementation method, concentration	Impact	Reference
Vitamin E	<i>In vivo</i> (200 IU/male per day for 12 months in diet)	Improved sperm concentration and SOD and GPx activity in testicular cells.	[93]
	<i>In vivo</i> (4 mL/ram intramuscular injection every two weeks for three months)	Enhanced semen volume, sperm motility, concentration, and viability, with a reduction in sperm abnormalities.	[94]
	<i>In vitro</i> (1 mg/mL in cooled semen extender)	Improved sperm motility, viability, and decreased sperm abnormalities in Awassi ram semen cooled at 5°C for 120 hours.	[114]
Melatonin	<i>In vivo</i> (54 mg subcutaneous ear implants), each implant containing 18 mg, were inserted behind the left ear during the nonbreeding season	Increased seminal and plasma melatonin levels, testicular size, and testosterone concentrations in Merino and Poll Dorset rams. Enhanced sperm concentration, viability, morphology, and DNA integrity.	[99]
	<i>In vivo</i> (36 mg subcutaneous injection under the ear) during mild heat stress (HS; scrotal neck insulation for 96 hours)	Improved testicular blood flow, sperm motility, morphology, viability, and acrosome integrity in Dorset rams exposed to testicular HS.	[76]
	<i>In vivo</i> (36 mg subcutaneous injection under the ear) during the nonbreeding season	Improved testicular blood flow, testosterone levels, and sperm quality parameters in Ossimi rams.	[100]
	<i>In vitro</i> (1 mM to semen extender)	Improved post-thaw sperm motility in Merino rams	[117]
	<i>In vitro</i> (0.05–0.2 mM added to semen extender)	Enhanced sperm motility, membrane integrity, mitochondrial activity, and antioxidant capacity. Reduced lipid peroxidation in cooled semen.	[118]
L-carnitine (LC)	<i>In vivo</i> (50 mg/kg/day) intravenous injection for 60 days	Increased sperm cell concentration, blood LH, and testosterone. Reduced sperm abnormalities in Awassi rams.	[103]
	<i>In vitro</i> (5 mM to semen extender)	Improved sperm motility, viability, membrane and acrosome integrity, and mitochondrial activity in cooled ram semen stored at 4°C for 48 or 96 hours. Reduced lipid peroxidation. Improved the pregnancy rate after AI.	[119, 120]
N-acetyl cysteine (NAC)	<i>In vitro</i> (0.5 and 0.75 mM to semen extender)	0.5 mM improved post-thaw sperm motility, viability, and membrane integrity. 0.75 mM reduced post-thaw sperm quality.	[121]

Antioxidants	Supplementation method, concentration	Impact	Reference
Lycopene	<i>In vitro</i> (2.5 µM lycopene added to the semen extender)	Enhanced sperm motility, viability, membrane and acrosome integrity, and mitochondrial potential in cooled Hu ram semen stored at 4°C for five days.	[124]
	<i>In vitro</i> (0.5 g/100 mL to the semen extender)	Improved post-thaw sperm motility, viability, and kinematics in Arabian rams.	[126]
	<i>In vitro</i> (0.1, 1, and 5 µM to the semen extender)	All concentrations had no immediate effect on post-thaw semen quality. However, 5 µM lycopene negatively impacted sperm motility after two hours of incubation in Santa Inês rams.	[128]
Coenzyme Q10 (CoQ10)	<i>In vitro</i> (2 µM to the semen extender)	Improved the post-thaw sperm motility and viability, and reduced the apoptotic-like changes and lipid peroxidation.	[131]

Source: Developed by the authors.

Table 1.

Impact of antioxidant supplementation (in vivo and in vitro) on fresh, chilled, and cryopreserved ram semen quality.

E supplementation) [93]. Furthermore, compared to the control group, the authors found that vitamin E greatly reduced the levels of malondialdehyde (MDA) and raised SOD and GPx activity in testicular cell membranes and mitochondria [93]. The effects of vitamin E administration in relation to the season on ram semen quality have also been investigated [94]. In the latter study, the authors noticed that intramuscular injection of vitamin E (4 mL/ram) every two weeks for three months enhanced semen volume, sperm motility, concentration, and viability, as well as reduced sperm abnormalities compared to the controls [94]. Another study done on Ossimi rams showed that the injection of each ram with 450 mg of vitamin E and 5 mg of sodium selenite twice per week for one month significantly improved semen volume, sperm motility, and concentration, along with reducing the percentages of abnormal sperm [95]. Furthermore, treated rams exhibited elevated serum testosterone, enhanced antioxidant capacity as indicated by an elevation in GPx, improved testes measurements, and reduced reaction time in libido [95]. On the contrary, the intramuscular injection of Barki rams with vitamin E (204 mg/ram) and selenium (4.5 mg/ram) together twice weekly for 64 days adversely influenced semen quality and antioxidant status [96] in comparison with the controls. This indicates that the effect of vitamin E and selenium on sheep semen quality depends on various factors, including the breed, the dosage, the duration, and the management systems.

5.1.2 Zinc

It has been shown that zinc (Zn) can affect reproductive potential by activating and maintaining the germinal epithelium of seminiferous tubules and boosting

testosterone production and secretion, which affects spermatogenesis [97]. Supplementing ram lambs' diets with 40 mg of organic Zn per kilogram of feed for 120 days improved testicular growth and sperm production [97]. Another study found that dietary supplementation of Zandi lambs with 40 mg/kg of Zn sulfate for 85 days significantly enhanced epididymal sperm total and progressive motility, membrane integrity, and viability compared to the control groups [98]. Also, the GPx activity and total antioxidant capacity (TAC) were higher, and MDA levels were lower in the seminal plasma of the Zn sulfate group [98]. High antioxidant capacity was indicated by raising the GPx activity [98].

5.1.3 Melatonin

Research demonstrates that exogenous melatonin (N-acetyl-5-methoxytryptamine) administration can enhance ram reproductive functions, particularly during the nonbreeding season or under environmental stress. Subcutaneous melatonin implants or injections elevated seminal and plasma melatonin levels, increased testicular size and blood flow, and raised testosterone concentrations in Merino [99], Poll Dorset [76, 99], and Ossimi [100] sheep (see **Table 1**). These changes were accompanied by improved semen production, including higher sperm concentration and enhanced sperm viability, morphology, and DNA integrity [99, 100]. Notably, recent studies showed that the slow-release of melatonin mitigated the negative impacts of HS on testicular blood flow, sperm motility, and morphology, and reduced oxidative stress and DNA fragmentation [76, 99–101]. Additionally, melatonin optimized endocrine profiles and metabolic pathways, including tryptophan metabolism in seminal plasma [101].

5.1.4 L-carnitine

L-carnitine (LC), a water-soluble amino acid, has been demonstrated to increase sperm motility by facilitating the transportation of long-chain fatty acids into the mitochondria to be metabolized by β -oxidation, leading to the production of high ATP [102]. It has been shown that the intravenous injection of Awassi rams with LC (50 mg/kg per day) for 60 days significantly increased sperm concentration ($3.41 \times 10^6/\text{mL}$ vs. $2.89 \times 10^6/\text{mL}$) and reduced the percentage of abnormal sperm (17.12% vs. 25.32%) compared to the controls in ejaculated semen samples (see **Table 1**). Additionally, treated rams showed higher levels of blood LH, testosterone, liver enzymes, and glucose [103].

5.1.5 Fatty acids

Additionally, the effects of adding unsaturated fatty acids (UFAs) to the diet on sheep's ability to reproduce and the quality of their semen were examined. According to a recent study, there are no apparent differences in sperm quality between rams fed on a diet supplemented with canola oil, sunflower seed oil, or lemur oil combined with antioxidants (vitamin E 500 IU + selenium 0.5 ppm) and those fed on a control diet without supplements [104]. However, another study reported that, compared to animals fed a high-ratio (10:1) omega-6 to omega-3 fatty acids, rams fed a low-ratio (4:1) diet for 10 weeks showed improvement in semen volume, sperm concentration, motility, membrane integrity, and a reduction in

sperm DNA damage [105]. This latter beneficial effect of fatty acids has been linked to the increased incorporation of omega-3 fatty acids, especially docosahexaenoic and eicosapentaenoic acids, into seminal plasma and sperm plasma membranes, which lowered the omega-6:omega-3 ratio in blood plasma, seminal plasma, and spermatozoa [105]. Moreover, feeding South African indigenous rams on a diet supplemented with 5% flaxseed oil and 4% ascorbic acid for 60 days resulted in an elevation in sperm motility, plasma membrane integrity, and embryo development rates after IVF compared to the control group or those that received either supplement alone [106].

5.1.6 Plant extracts

Oral supplementation of Barki rams with *Moringa oleifera* leaf extract (40 mg/kg per day) notably improved semen volume, sperm concentration, and enhanced the activities of essential antioxidant enzymes in seminal plasma, such as CAT, GPx, and SOD, in addition to an increase in TAC and ascorbic acid levels [96]. At the same time, post-thaw sperm quality was also heightened, along with greater motility, viability, membrane integrity, and fewer defective acrosomes, DNA fragmentation, and MDA levels [96]. In a different study, Rahmani rams were given oral moringa oil (MO) and its nano-emulsion (NMO) for four months, and both treatments showed an improvement in some blood health markers, such as mean corpuscular hemoglobin, and a decline in aspartate transferase and total cholesterol [107]. Notably, MO supplementation significantly enhanced semen quality, increased mass motility, viability, membrane integrity, and sperm concentration, along with elevated serum and seminal testosterone levels [107]. Furthermore, sperm CAT gene expression was upregulated, and lipid peroxidation was reduced in the MO group, indicating the antioxidant activity of MO. However, NMO treatment showed mixed effects, including increased apoptotic marker caspase-3 expression and altered antioxidant gene regulation [107]. These results indicate that MO, particularly in its conventional form, holds promise as a natural supplement for improving reproductive health and semen quality in rams.

Moreover, another study [108] from our group evaluated the impact of dietary supplementation of peanut skin (PS) on ram semen quality and proved that the presence of natural polyphenols in PS was crucial for maintaining semen quality. In this study, Florida Native lambs were fed diets supplemented with 20% untreated (rich in polyphenols) or treated (without polyphenols) PS for 42 days. We found that while scrotal circumference, testicular and epididymal morphometrics, and sperm concentration were similar between the two groups, sperm motility, viability, membrane integrity, and acrosome integrity were significantly lower in the treated PS group, meanwhile, it was the opposite for sperm abnormalities [108]. These results indicate that the polyphenols naturally present in the PS can preserve sperm functions, and their removal negatively impacts semen quality. These findings underscore the importance of polyphenols as dietary supplements in supporting male fertility.

To sum up, *in vivo* administration of antioxidants, including vitamins E, selenium, zinc, melatonin, LC, fatty acids, and plant-based extracts like moringa and PS, offers a robust strategy for enhancing sheep semen quality and consequently their reproductive resilience. These compounds counteract oxidative stress induced by seasonal, thermal, and metabolic challenges through their ability to boost

endogenous antioxidant defenses. However, as demonstrated by inconsistent results among research studies, their efficacy is largely contingent on the correct dosage, form, and physiological conditions of the animal. The usefulness of strategic *in vivo* antioxidant supplementation in modern sheep breeding and management systems is ultimately reinforced by the fact that it not only increases spermatogenesis and semen quality but also improves the ram's ability to retain fertility under substandard circumstances.

5.2 *In vitro* antioxidants

During semen cooling and freezing, the significant overproduction of ROS can overwhelm the endogenous antioxidant defense systems, diminishing their protective capacity [87, 109]. Consequently, supplementing exogenous antioxidants is a key strategy in semen preservation techniques to mitigate the detrimental effects of excessive ROS on spermatozoa [109].

5.2.1 Enzymatic antioxidants

Enzymatic antioxidants such as SOD, CAT, GPx, and GR are molecules that shield the cells from the damage caused by ROS [110]. They achieve this by boosting systems that either directly neutralize ROS or generate extra antioxidants, preserving cell integrity [110]. Previous studies showed that supplementing semen extender with 800 U/mL SOD improved sperm motility in chilled ram spermatozoa [111]. Moreover, another study demonstrated that supplementing ram semen extender with 100 U/mL SOD or 2 and 5 mM glutathione (GSH) effectively preserved the acrosome in frozen/thawed sperm, while SOD specifically protected sperm membranes and supported higher *in vivo* fertilization rates compared to GSH [112]. Furthermore, compared to controls, ewes inseminated with cooled semen treated with a combination of SOD (800 U/mL) and CAT (200 U/mL) and kept for up to 14 days at 5°C had higher conception rates [111]. However, higher concentrations of CAT (400 and 800 U/mL) proved to be toxic to the sperm [113].

5.2.2 Non-enzymatic antioxidants

Lipid peroxyl and alkoxyl radicals are efficiently neutralized by chain-breaking antioxidants, including ubiquinol and vitamins C and E [89]. Prior research has demonstrated that adding vitamin C (0.9 mg/mL) or vitamin E (1 mg/mL) to the semen extender enhanced sperm motility and vitality, and reduced sperm abnormalities in semen cooled at 5°C for 120 hours in Awassi rams, as compared to the non-supplemented group [114]. In cryopreserved ram semen, it has been found that post-thaw sperm motility, viability, and membrane integrity significantly improved with vitamins C and E supplementation, particularly with 7 mM vitamin C, which yielded the highest motility [115]. Contrary to these observations, some studies did not find significant improvements in ram semen quality treated with vitamins C and E during liquid cold storage [116]. The differences could be ascribed to the sheep breed, types of extenders used, and the concentrations of vitamins C and E.

Melatonin, a strong antioxidant, is well-known for its ability to shield sperm from damage caused by ROS in cooled and frozen ram semen. Melatonin acts as a powerful

detoxifier for different ROS and reactive nitrogen species (RNS), as well as a hydroxyl radical scavenger. Melatonin supplementation (see **Table 1**) has been shown to improve the quality of cryopreserved and cooled ram semen on multiple occasions [75, 117, 118]. For example, it has been demonstrated that in frozen/thawed Merino ram semen, adding 1 mM melatonin to the semen extender enhanced post-thaw sperm motility in comparison to the control [117]. Lower melatonin concentrations (0.05–0.2 mM) improved sperm motility, membrane integrity, mitochondrial activity, and antioxidant capacity while lowering lipid peroxidation in ram semen that had been stored at 4°C for five days [118]. Melatonin not only has a major impact on the quality of frozen/thawed semen obtained from healthy rams, but it also improved the freezability and quality of semen collected from rams exposed to HS [75]. These findings highlight the crucial role played by melatonin in safeguarding the semen against oxidative stress occurring during cooling and cryopreservation procedures.

Various amino acids are known to enhance sperm cryotolerance and mitigate the deleterious effects exerted on sperm by cold shock due to cooling and cryopreservation [102]. For instance, it has been demonstrated that supplementing semen extender with 5 mM LC improved sperm motility, viability, membrane integrity, acrosome integrity, and mitochondrial activity in cooled ram semen stored at 4°C for 48 or 96 hours (see **Table 1**) [119, 120]. In the meantime, it reduced lipid peroxidation [119]. LC not only improved semen quality but also enhanced sperm fertility in sheep. A study conducted on sheep AI reported that the pregnancy rates were higher in the ewes inseminated with cooled semen treated with 5 mM LC than those in the controls [120].

The thiol-containing substance N-acetyl cysteine (NAC) is a strong antioxidant and a free radical scavenger. It has been demonstrated that NAC increases the quantity and stability of sperm plasma membrane proteins during chilling or cryopreservation and inhibits their sedimentation [89]. Additionally, it has been documented that NAC supplementation can improve the quality of frozen/thawed ram semen [121]. The findings demonstrated that, among the different concentrations used, 0.5 mM NAC supplementation to a skim milk–egg yolk–glycerol-based extender enhanced sperm motility, viability, and membrane integrity in frozen/thawed ram semen as compared to those supplemented with 0.75 mM NAC (see **Table 1**) [121].

Lycopene, a strong antioxidant, belongs to the tetraterpene carotenoid family. It has been recognized for its red appearance, having 13 double bonds that are arranged linearly. Lycopene is the most efficient anti-radical molecule among carotenoids, which emphasizes its biological functions [122, 123]. Lycopene is found in many fruits and vegetables, including tomatoes, papayas, watermelons, apricots, pink grapefruits, and rosehips [122, 123]. Several studies have reported the positive impacts of lycopene supplementation on the quality of chilled ram semen. For example, adding 2.5 μ M lycopene to the semen extender enhanced sperm viability, motility, membrane and acrosome integrities, mitochondrial potential, and antioxidant activity in Hu ram semen stored at 4°C for five days (see **Table 1**) [124]. Other studies reported that 0.5 mM lycopene supplementation increased sperm motility and mitochondrial activity in chilled Merino ram semen kept for 72 hours [125]. Lycopene's protective properties were also observed in cryopreserved ram semen. In Arabian rams, it has been found that lycopene supplementation at 0.5 g/100 mL increased post-thaw motility, viability, and kinematic characteristics (see

Table 1) [126]. Similarly, compared to controls, 3 mg/100 mL of ascorbic acid and lycopene greatly increased post-thaw motility in Sapudi rams [127]. However, following a two-hour incubation period, lycopene adversely affected progressive motility and motion patterns in Santa Inês rams, while it had no immediate influence on post-thaw semen quality (see **Table 1**) [128]. This implies that the concentration of lycopene employed, the circumstances of preservation, and the duration of incubation can all affect the quality of sperm.

Coenzyme Q10 (CoQ10), a part of the respiratory chain in mitochondria, plays an important role in energy metabolism and has antioxidant properties in cell membranes [129]. It has been shown that the testis produces substantial quantities of CoQ10 [130]. Moreover, CoQ10 reduced form ubiquinol is demonstrated in sperm and serves as an antioxidant [129]. Incorporating 2 μ M CoQ10 into semen extender enhanced post-thaw sperm motility and viability, and it reduced apoptotic-like alterations and lipid peroxidation in rams, which resulted in an increase in reproductive potential (see **Table 1**) [131].

Various plant extracts have been demonstrated to have advantages in protecting ram semen during preservation due to their antioxidant capacities. For instance, phenolic acids, flavonol glucosides, phenolic volatile oils, and tannins are abundant in clove (*Syzygium aromaticum*) and act as powerful metal chelators and free radical scavengers [132]. Clove extracts at concentrations of 35–75 μ g/mL-maintained ram sperm motility, viability, and membrane integrity both during cooling and after freezing and thawing [132]. Also, green tea (*Camellia sinensis*) extracts at 10 mg/L-improved post-thaw ram sperm viability, motility, and antioxidant capacity, whereas higher levels induced oxidative damage [133]. This was ascribed to the high content of polyphenols, catechins, and epigallocatechin gallate in green tea, which have potent free radical scavenging activity. Other plant extracts, including *Foeniculum vulgare* (fennel) at 10 mg/mL [134] and *Entada abyssinica* at 375 μ g/mL [135], also improved post-thaw ram sperm quality and diminished lipid peroxidation. Moreover, adding 5–10 mg/L of *Zingiber officinale* (ginger) to the semen extender enhanced the frozen/thawed sperm viability, acrosome integrity, and mitochondrial activity in rams [136]. On the contrary, rosemary [137] and pomegranate [138] showed adverse effects at larger doses, highlighting the significance of using optimum concentrations of the extracts. Overall, these findings show that plant-derived antioxidants can successfully reduce damage caused by cryopreservation in ram sperm, with their effectiveness being correlated with the bioactive compounds and concentrations employed.

In summary, the strategic supplementation of exogenous antioxidants, including enzymatic antioxidants, vitamins, melatonin, amino acids, lycopene, CoQ10, and plant-based extracts, has proven to be an essential approach to counteracting the oxidative stress induced by semen cooling and cryopreservation in rams, with efficacy being highly dependent on compound type, concentration, and preservation conditions.

6. Conclusions and future implications

Ram semen quality is a multifaceted trait influenced by a complex interplay of intrinsic factors such as age, breed, and genetics, and extrinsic factors like seasonality, nutrition, HS, and semen handling. Oxidative stress emerges as a central,

unifying challenge across these factors, directly impairing sperm motility, membrane integrity, DNA stability, and overall functions and fertility. Herein, we underscore that both *in vivo* and *in vitro* antioxidant supplementations offer promising, evidence-based approaches to mitigate this damage. Ultimately, enhancing ram reproductive performance requires an integrated management approach that combines optimized breeding selection, strategic nutrition, careful handling of semen, and the judicious use of antioxidants. By addressing oxidative stress at its source and supporting the ram's endogenous defenses, producers can improve semen cryosurvival, AI success rates, and overall flock fertility, which are key steps toward meeting growing demands in sheep production. Looking forward, natural plant-based antioxidants hold great promise for improving ram fertility in an affordable and sustainable way. However, more practical research is needed. Future work should focus on finding the best doses and combinations of plant-based extracts for specific breeds and farm conditions, avoiding the "one size fits all" approach. It is also imperative to use local plants and farm byproducts like peanut skins or fruit peels, which can be a low-cost source of antioxidants. With climate change challenges, studies should focus on how well these plant compounds can protect rams and their semen from the deleterious effects of HS. Finally, farmers need clear, simple guidelines on how to use these natural additives effectively, which will make sheep farming more resilient, productive, and sustainable.

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Conflict of Interest

The authors declare no conflict of interest.

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Section 4

Animal Health

Chapter 6

Skin Diseases and Their Impact on Sheep Production

Daniel Thomas Yoksa, Raymond Habila Gapsiso, Yusuf Jairus and David Dan Andeze

Abstract

Sheep farming is a lucrative business in Nigeria; the rams are used as food and sacrificed during religious festivities. Trading hides and skin is a source of employment for many people at both local and international levels. The overall health status of an animal can be judged by how the skin looks at the point of sale. Cutaneous lesions in sheep are a major setback to this business, and there is a need to understand the causative agents and provide preventive measures to boost sheep farming. Many etiological agents, ranging from parasites, bacteria, fungi, viruses, and mineral deficiencies, have been implicated in causing cutaneous lesions in sheep farming. Decreased market value of animals, rejection of hides at tanneries, and sometimes partial or total condemnation at slaughterhouses are major setbacks to sheep production globally. Zoonotic risks have also been identified in some parasitic and bacterial causes of cutaneous lesions. Treatment of cutaneous lesions in sheep production is not only expensive but also time-consuming. Preventive measures are the only solution to improve sheep productivity for better profit.

Keywords: cutaneous lesions, mange, dermatitis, sheep pox, deficiency, alopecia

1. Introduction

In Nigeria, the estimated population of sheep is 47.9 million [1]. This population is mainly kept to serve as sources of food and employment for a significant group of people, including farmers, marketers, butchers, grillers, and food vendors, and is also used during religious festivities [2, 3]. Hide and skin trading is a lucrative business at both domestic and international levels. The marketability of an animal depends on how healthy the animal looks at the point of sale, and the skin plays a vital role in representing the overall health status of the sheep. Skin diseases of sheep can be caused by different etiological agents such as parasites (ticks, lice, fleas, and mites), bacteria (Streptothricosis, Caseous lymphadenitis), viruses (Orf virus), and nutritional conditions (deficiency of vitamin A, selenium, zinc, etc.) [4]. Substantial economic losses have been documented in terms of poor wool quality resulting from intense pruritic wounds, which pose great discomfort, decreased milk yield in

lactating does, slow growth rates resulting in lighter weight, and decreased fertility [5]. Cutaneous lesions can be classified based on their morphological appearance as ulcerative, scabs, crusts, alopecia, and papules. Crusts and alopecia are often seen with fungal infections, while pruritic dermatitis is seen with ectoparasitic infestation [6]. Understanding the etiological agents and the pathophysiology of skin diseases in sheep production is important for a better diagnostic and treatment approach, which will have a positive effect on improving productivity.

2. Methodology

This manuscript is a handy tool for young and practicing veterinarians, students, and animal health practitioners who are interested in sheep farming and dermatology. Search engines such as Google, along with other veterinary textbooks and field experiences, were combined to obtain a detailed summary about the etiological agents, modes of entry, and economic impact of such skin diseases on overall sheep production.

3. Results

3.1 Parasitic causes of skin diseases in sheep production

Several parasites are known to parasitize the skin of sheep, there by leading to injuries ranging from acute to chronic inflammatory conditions, allergic reactions, and sometimes hypersensitivity conditions, causing gross, intensely pruritic wounds that can sometimes be difficult to treat.

3.1.1 Mange

In sheep, four primary types of mange mites are seen: sarcoptic, chorioptic, psoroptic, and demodectic mites. Mites can cause serious cutaneous damage, resulting in financial losses for both domestic and food animals. Most species of *Demodex* mites survive their whole life cycle in the lumens of hair follicles or sebaceous glands as part of the typical normal fauna of the skin of most mammals. Skin lesions of demodectic mange only appear when the normal balance between the parasite and the host is altered to favor the mite's growth, causing skin lesions of demodectic mange to be produced. Thus, identification of large numbers of adult mites or an increased number of immature mites in skin scrapings or biopsy samples is required for the diagnosis of demodicosis [7]. The scabies mite (*Psoroptes ovis*) burrows into the skin of its host, causing varying degrees of dermatitis, a condition known as sarcoptic mange. Although cases of sarcoptic mange in goats often resolve themselves without developing severe signs, heavily infested sheep may exhibit crusty lesions and extensive hair loss around the muzzle, eyes, and ears; lesions on the inner thighs extending to the hocks, brisket, underside, and axillary region; dermal thickening and wrinkling on the scrotum and ears; and dry, scaly skin on all parts of the body, especially in areas of hair loss [8]. Mange mites affect different ages of sheep, especially those kept under poor management conditions. Spread between ewe and lamb occurs during suckling. Infections are a major welfare concern because of the debilitating nature of the disease and its association with high morbidity and high economic losses caused by decreased growth rates [9]. Zoonotic

risks have also been associated with this condition. **Figure 1** Shows the photomicrograph of a sheep mite.

3.1.2 Lice

Lice are among the major causes of cutaneous lesions in sheep production worldwide. They cause great economic losses in terms of decreased production and rejection of hides at tanneries [10]. It has been reported that all species of lice cause skin irritation, which triggers itching, licking, and restlessness among the sheep population, resulting in decreased skin quality and an overall effect on production [10]. Sheep may become infested with one or more species of the chewing lice of the genus *Bovicola* (a known species of economic importance is *B. ovis*) or the blood-sucking lice of the genus *Linognathus* (*Linognathus pedalis*, *Linognathus ovillus*, and *Linognathus africanus*) [11]. **Table 1** presents the common lice of sheep and their predilection sites.

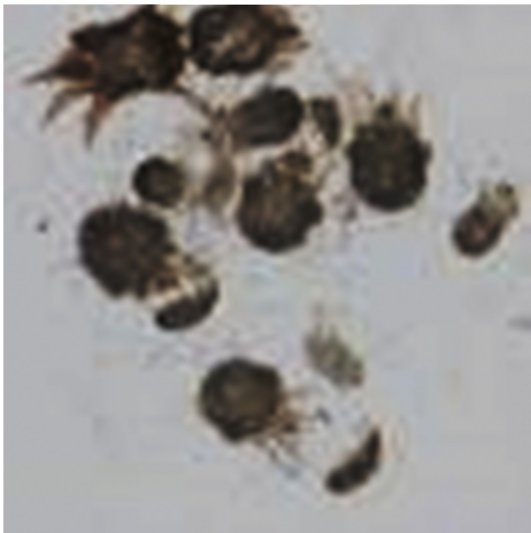


Figure 1.
Psoroptes ovis. Source: MSD Merck's Manual Online Edition.

Species	Type of lice	Common name	Predilection site
<i>Bovicola ovis</i>	Chewing	Ssheep biting louse	Dorsum of sheep may also extend cranially or caudally
<i>Linognathus pedalis</i>	Blood sucking	Ssheep foot louse	Except for heavy infestation, they are usually found on the hairy part of the foot
<i>Linognathus ovillus</i>	Blood sucking	Sheep face and body louse	hairy parts of the skin may spread to other parts with heavy infestation
<i>Lignognathus africanus</i>	Blood sucking	African blue louse	found on the back, rib, loin, and shoulder areas

Source: developed by the authors.

Table 1.
Lice of sheep: common names and predilection sites.

3.1.3 Flea

In sheep production, itching, wool loss, and severe skin damage can be caused by flea infestation. The sticktight flea (*Echidnophaga gallinacea*) is the most common flea affecting sheep and goat production. They remain attached to their host, sometimes for two to three weeks, feeding and laying eggs. Heavy infestation can cause severe ulcers, especially in the head region [12]. Spread to other animals occurs after the eggs transform into larvae on the ground. Spread to humans has also been reported with heavy flea infestation in sheep [12]. **Figure 2** below shows the photomicrograph of sticktight flea of sheep.

3.1.4 Ticks

Ticks can cause severe cutaneous lesions in sheep either through the direct effects of their attachment and bites or indirectly by serving as mechanical vectors for diseases such as tick-borne fever, tick paralysis, anaplasmosis, and babesiosis. Two important families of ticks are Ixodidae (hard ticks) and Argasidae (soft ticks) [13]. Direct losses to hide and skin quality, severe anemia due to blood loss from heavy infestation, and allergies caused by toxins and saliva during feeding can trigger immunological reactions that may be detrimental to the animal [13]. Indirect economic losses are due to the high cost of treatments, contamination of the environment by tick control chemicals, and disease transmission [13].

3.1.5 Flies and sheep ked

Sheep ked (*Melophagus ovinus*) is a widely common and important ectoparasite of sheep. Keds are wingless, reddish to brown in color, and are covered with short bristly hair [14]. They pierce the skin with their mouthparts to suck blood and are usually found on the shoulder, neck, flank, breast, and sometimes on the rump,



Figure 2.
Sticktight flea. Sourced: MSD Merck's Manual Online Edition.

where dust and other debris accumulate; however, keds are not typically found on the back. Their bites most often trigger intense itching on most parts of the body, and sheep are often seen biting and scratching the affected areas, which causes wool damage [14]. The grade and value of the skin are greatly reduced by the brown discoloration caused by the excrement of the parasite. Poor weight gain and decreased wool production also occur [15]. A skin defect called cockle is also a result of ked infestation. Sheep lose blood from heavy infestations, which can lead to anemia. Transmission of the nonpathogenic protozoan parasite of sheep (*Trypanosoma melophagium*) is also a sequela of sheep ked infestation [14]. Shearing and other management practices have been advocated as preventive measures [16]. **Figure 3** presents the photomicrograph of a sheep ked.

3.2 Bacterial causes of skin diseases in sheep production

Bacteria are also a major cause of skin diseases in sheep production; they cause diseases either as secondary invaders following injury to the skin via parasites or poor husbandry practices that cause unnecessary stress [16].

3.2.1 Dermatophilosis

This is a bacterial disease of the skin that is caused by *Dermatophilus congolense*. The disease occurs most commonly in young and immunocompromised animals. For the disease to occur, the bacterial zoospores must reach the skin barrier where the defense is lowered or compromised [17]. The disease is an exudative skin condition that is characterized by inflammation and matting of the wool. Skin lesions are seen on the dorsal midline and can spread laterally [18]. Direct wool production losses have been attributed to dermatophilosis. Blowfly strike has also been reported to occur because of the condition. Substantial economic losses, coupled with the challenges encountered in control measures, have made dermatophilosis an important disease [18].



Figure 3.
Sheep ked (Melophagus ovinus). Source: BioQuip.

3.2.2 Other bacterial skin disease

Other bacteria causing skin infections include *Staphylococcus aureus* and *Staphylococcus intermedius*, which causes Staphylococcal dermatitis in sheep, leading to a variety of boils, abscessation, and several skin conditions, thereby decreasing the quality and monetary value of the wool, hide, and skin [19].

3.3 Fungal causes of skin diseases in sheep production

Fungi, microbes common in the environment and as microflora of mucosae, exist as yeasts or as branched filamentous pseudohyphal or hyphal forms (molds) [7]. Fungi contain a variety of complex molecules that are arranged to form cell walls and capsules, which aid in the colonization of tissues and protect the microbe against phagocytosis and other defense mechanisms [7]. The physical properties of the skin and its keratin content make it a suitable environment for keratinolytic and keratinophilic fungi colonization [20]. Respiratory infections have also been reported in tannery workers from fungi-infected sheep skin [20].

3.3.1 Cutaneous mycoses

Cutaneous mycoses, also called superficial mycoses, are infections of cornified tissue, which include claws, hair, and the topmost part of the skin layer (epidermis). The fungi are rarely found in the dermis or subcutis, but tissue destruction and host response can be extensive [7]. Dermatophytosis, cutaneous candidiasis, and *Malassezia* dermatitis are known to cause diseases in domestic animals [7]. Dermatophytes are classes of fungi that cause skin lesions in domestic animals. They cause infections worldwide. The most important pathogenic genera are *Epidermophyton*, *Microsporum*, and *Trichophyton*. Fungal species that more commonly infect domestic animals are included in the genera *Microsporum* and *Trichophyton*. *Epidermophyton* is adapted to human beings (anthropophilic) and rarely infects animals [21]. Superficial and cutaneous mycoses (dermatophytosis) are acquired by contact with infected animals or by contact with infective material such as shed scales or hair in the environment or on fomites (e.g., combs, brushes, clippers, equine tack). They are more contagious than other fungal infections, are more common in hot, humid environments, and young animals are more susceptible than adults [22]. Animals kept in overcrowded, dirty, or damp areas and those with inadequate nutrition, or those that are immunosuppressed are also more susceptible [7]. **Figure 4** presents the classical appearance of a ringworm in sheep.

3.4 Viral causes of skin diseases in sheep production

Viruses are known to infect sheep and cause diseases manifesting as cutaneous lesions. Several economically important families and genera of viruses belong to this category.

3.4.1 Contagious ecthyma in sheep

Contagious ecthyma (Pustular dermatitis, Orf, Sore Mouth) is caused by a parapox virus. It is a disease of young animals. Transmission of the virus occurs



Figure 4.
*Ringworm in sheep (*Trichophyton spp.*). Source: Sargison, N.D. (2002).*

through contact. The virus is resistant to desiccation in the environment, and the virus can be recovered from scabs for as long as 12 years [23]. Lesions are found on the commissure of the mouth and can sometimes spread to the ears and cheeks. Scab lesions can sometimes coalesce to form large scabs, decreasing the marketability of the affected sheep due to cutaneous lesions. Secondary bacterial infections, especially with *Dermatophilus congolensis*, have also been reported, with lesions referred to as strawberry foot. Extensive lesions on the foot can sometimes lead to lameness, which can further affect the overall performance of the animal [23]. The **Figure 5** below presents scab lesions from contagious ecthyma.

3.4.2 *Sheep pox*

Sheep pox is caused by a poxvirus of the genus Capripoxvirus, belonging to the family Poxviridae. It is a highly contagious disease of small ruminants, affecting all



Figure 5.
Sheep with scab lesions at the commissure of the mouth. Source: developed by the authors.

Mineral	Uses	Lesions
Zinc	Keratinization and immune function	Hair loss, Abnormal hoof, Hyperkeratosis
Vitamin A	Maintenance of epithelium	Alopecia, scaling, and crusting of the skin
Vitamin E	Antioxidant preventing skin cells from damage	Dry and flaky skin, Impaired skin healing
Riboflavin	Growth and development	Dark patches on the wool

Source: developed by the authors.

Table 2.
Minerals their uses and lesions on the skin.

ages of both wild and domestic small ruminants [24]. Substantial economic losses in the productivity of animals with the disease have been reported. Decreased milk production and lesions also cause damage to hides and wool, generally affecting the trade of such animals [24]. Sheep pox has been reported to be the main cause of skin downgrading in Ethiopia [25].

3.5 Nutritional causes of skin diseases in sheep production

Deficiency or excesses of minerals in sheep farming can cause skin lesions, and this can affect the overall performance of the animal, decreasing its market value. Some of these mineral deficiencies are listed below in **Table 2**.

4. Conclusion(s)

Lesions on the skin have a great economic impact on sheep production, ranging from rejection at tanneries, decreased marketability of the affected animal, overall rejection at slaughter, and decreased general performance of the animal's productivity. Understanding the etiologies of those cutaneous lesions in sheep production is important in planning a preventative approach for better, more profitable production.

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Conflict of interest

We have no conflicts of interest to declare.

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The resilience of animals is of decisive importance in all life functions. Several environmental factors, including the type and nutrient content of the feed, the feeding process, animal health, and breeding and environmental factors, influence this resilience. Of these, stress plays a prominent role, affecting all physiological functions, including resilience and production efficiency, the development of potential diseases, and even sperm quality, which is crucial for reproduction. One of the basic elements of stress is the feeding environment, which includes the potential for GHG reduction and the use of succulents and aquatic plants for nutrition. Within reproductive biology, semen production and quality, as well as the factors influencing it, are of essential importance, especially in the case of the use of artificial insemination. In addition to meat, milk, and wool, the usability of the skin is of fundamental importance, and together they determine the economics of production. The treatment and prevention of factors that influence skin quality (parasites, bacteria, viruses, nutrient deficiencies, etc.) are fundamental for economic utilization.

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