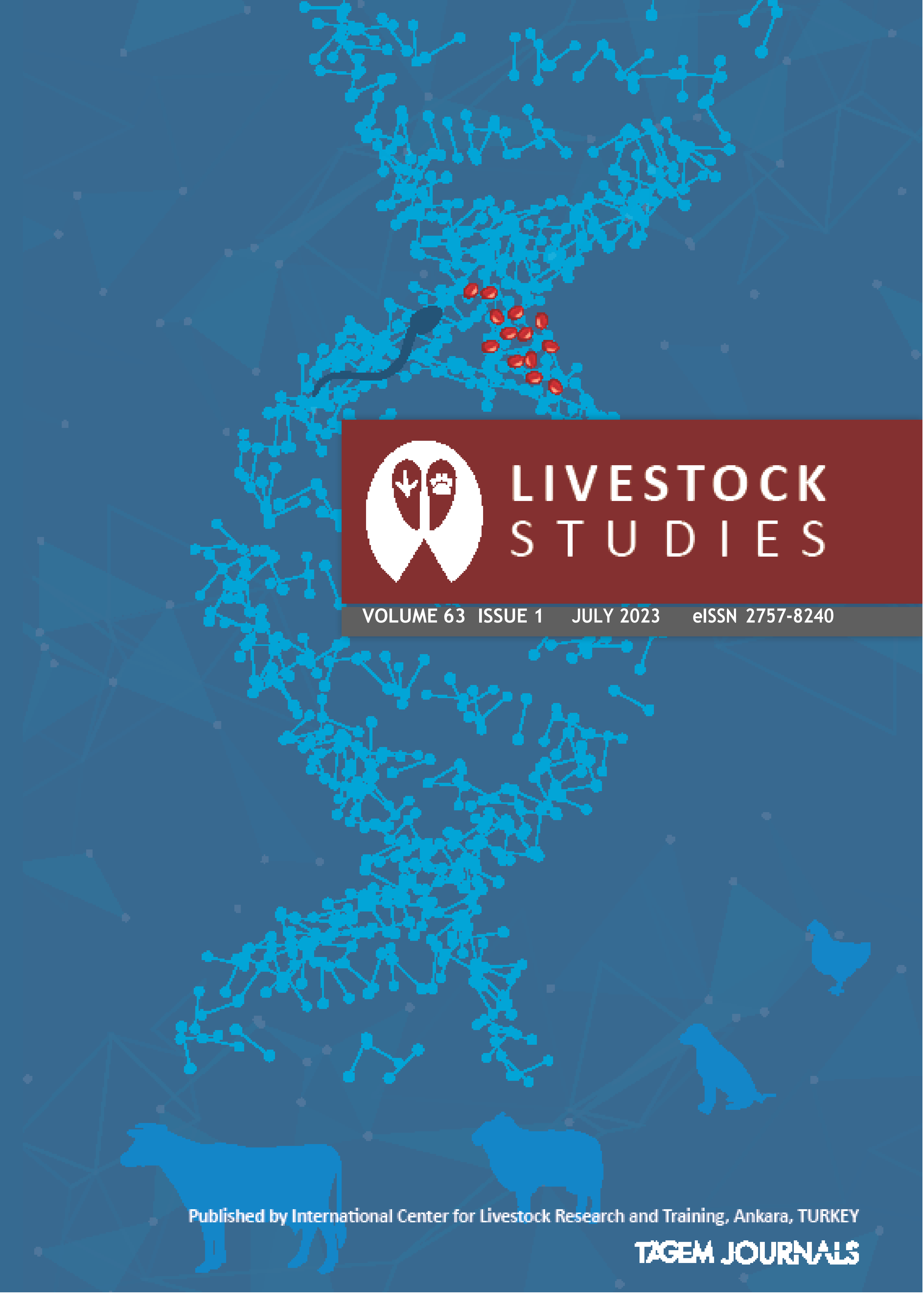

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Our Institute Lalahan International Center for Livestock, Research and Training has been operating in the field of Animal Science and Livestock since 1951. Among the livestock activities, our Institute continues its activities in the fields of cattle breeding, ovine breeding and poultry breeding. In addition Institute's Breeding, Animal Nutrition, Genetics, Artificial Insemination and Embryo laboratories actively serve. Numerous research projects have been completed or still continue to be carried out in these areas. Institute has a journal named "Lalahan Livestock Research Institute Journal" which has been publishing 2 issues per year since 1959. The journal has the status of a National Refereed Journal followed by ULAKBİM (Turkish Academic Network and Information Center) in the field of Livestock. The journal, which has a strong archive and knowledge in its field, will continue its publication in English in order to carry it to International Standards. The journal will continue its publishing life as its new name 'Livestock Studies'.

Livestock Studies covers all kind of studies related to farm animals from poultry and bees to cattle, sheep, goats, etc. as follows:

Livestock Studies has been monitored: ULAKBIM (Turkish National Academic Network and Information Center),FAO AGRIS, CAP Abstract, CABI Full Text, Animal Breeding Abstracts, Google Scholar.

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REVIEW ARTICLE

Pandemics and Ecological Animal Husbandry

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Abstract

The pandemics to which humanity has been subjected throughout history will also continue to exist in the future. There may be many reasons for the development of pandemics and threats to human and animal health. Whatever the reason, previous pandemics and also this current Covid-19 period revealed the importance of ecological agricultural production. The destruction of natural habitats, industrial agriculture, industrialization, and the decrease in biodiversity disrupt the ecological balance and create an environment for pandemic formation, endangering both human and animal health. This review is focusing on the importance of ecological animal husbandry in terms of both human and animal health and welfare, and especially understanding after the pandemics are part of our life.

Introduction

World Health Organization (WHO) claims that "A pandemic is the worldwide spread of a new disease" (WHO, 2020a). However, some confusion and contradictory situations have arisen from time to time in the use of the term "pandemic". While some claimed that a certain level of high contagiousness is sufficient to report a pandemic, others have argued that the severity of infection should also be taken into account (Morens *et al.*, 2009). For an infection that has turned into a disease to become a pandemic; an epidemic that has not been exposed before must emerge, the disease factor must be transmitted to people, and cause a dangerous disease, and, the cause of the disease must be able to spread easily and continuously. The prevalence of a disease or condition that causes the death of a large number of people is not enough to be qualified as a pandemic, it must also be contagious (Aslan, 2020). Especially in the last 30 years, there has been an increase in communicable diseases, more than 70% of which are zoonotic (Wang and Crameri, 2014). Zoonotic diseases can be caused by viruses, bacteria, parasites, and mycetes. Sometimes, animals can seem healthy even if they carry factors that can infect people due to zoonotic disease. It is estimated that more than six out of every ten infectious diseases known to occur in humans can be spread by animals. Three out of every four new infectious diseases seen in humans are caused by animals (CDC, 2017). With the effects of globalization, the time taken for communicable diseases to spread throughout a regional area has become much shorter due to the increasing population, population density, and mobility. Globalization in trade, increasing population mobility, and international travel are the main human influence on the emergence, re-emergence and transmission of infectious diseases in the twenty-first century (Bickley *et al.*, 2021). Lastly, according to WHO, Covid-19 emerged in China on December 31, 2019; in Thailand on January 13, 2020, in America on January 21, 2020, immediately after China, and then rapidly all over the world (Budak and Korkmaz, 2020). Countries closing their borders as a precaution to prevent the spreading of Covid-19 has shown the effect of globalization on the epidemic diseases becoming a pandemic.

Among the major pandemics in world history (Table 1), the Plague of Justinian (causative agent Yersinia pestis, 541-542) which caused more than a quarter of the world's population (30-50 million people; The Black Plague (Black Death, 1347) caused the death of 30-60% of the European population (75-200 million people), Smallpox (poxvirus) which emerged in the fifteenth century killed tens of millions of people, and maintained its contagiousness until 1980 when WHO announced its eradication can be stated (Roos, 2020).

Covid-19

Due to this new coronavirus, which emerged in Wuhan, China at the end of 2019 and was classified as a pandemic by the WHO, over 455 million cases and 6 million deaths were detected until 15.03.2022 globally (WHO, 2022).

Over 60% of current and developing pathogens that affect people originate from animals and 75% of them are from wildlife, so human health, animal health, and ecosystem health must be considered together (WHO, 2018). The destruction of forest areas, the decrease in biodiversity, the excessive use of pesticides and chemical fertilizers due to industrial agriculture, intensive industrialization, and peoples lack of environmental awareness lead to the climate crisis (Aguirre, 2017). Main human threats to biodiversity are; overuse of species, habitat destruction, and the introduction of exotic species, all of which lead to ecosystem degradation that leads to changes in disease transmission patterns. Pathogen pollution, global toxicity, and global environmental changes due to climate increase the loss of biodiversity tremendously. Perhaps the most insidious factor out of these factors is climate change, which has a profound impact on all ecological processes, including some increased rise and drought in others. Increased coastal zone erosion with rising sea levels, increased tsunamis, hurricanes, and tropical storms, and the inability of many species to adapt to relatively rapid changes in climate regimes could potentially lead to mass extinctions (Aguirre, 2017). The pressure of human settlement and agriculture on natural ecosystems results in the expansion of ecotones where species from different habitats come together. This situation provides new

Table 1: Some of the major pandemics that have occurred over time (LePan, 2020; WHO, 2022).

Name	Time period	Type / Pre-human host	Death toll
Antonine Plague	165-180	Believed to be either smallpox or measles	5M
Japanese smallpox epidemic	735-737	Variola major virus	1M
Plague of Justinian	541-542	Yersinia pestis bacteria / Rats, fleas	30-50M
Black Death	1347-1351	Yersinia pestis bacteria / Rats, fleas	200M
New World Smallpox Outbreak	1520 – onwards	Variola major virus	56M
Great Plague of London	1665	Yersinia pestis bacteria / Rats, fleas	100.000
Italian plague	1629-1631	Yersinia pestis bacteria / Rats, fleas	1M
Cholera Pandemics 1-6	1817-1923	V. cholerae bacteria	1M+
Third Plague	1885	Yersinia pestis bacteria / Rats, fleas	12M (China and India)
Yellow Fever	Late 1800s	Virus / Mosquitoes	100.000-150.000 (U.S.)
Russian Flu	1889-1890	Believed to be H2N2 (avian origin)	1M
Spanish Flu	1918-1919	H1N1 virus / Pigs	40-50M
Asian Flu	1957-1958	H2N2 virus	1.1M
Hong Kong Flu	1968-1970	H3N2 virus	1M
HIV/AIDS	1981-present	Virus / Chimpanzees	25-35M
Swine Flu	2009-2010	H1N1 virus / Pigs	200.000
SARS	2002-2003	Coronavirus / Bats, Civets	770
Ebola	2014-2016	Ebolavirus / Wild animals	11.000
MERS	2015-Present	Coronavirus / Bats, camels	850
COVID-19	2019-Present	Coronavirus-Unknown (possibly pangolins)	6M (till March 15, 2022)

opportunities for pathogen spread, genetic diversification, and adaptation (Jones *et al.,* 2013). In The Global Climate in 2015-2019 report of the World Meteorology Organization (WMO), it was stated that the CO_2 emission resulting from the use of fossil fuels in the atmosphere has increased since 2015 and this rate of increase is 20% more than 2011-2015 period. Again, between the years 2015-2019, global warming increased by approximately 0.2 ^oC compared to the 2011-2015 period and reached 1.1 ^oC above the pre-industrial period (WMO, 2019). According to the report of the International Panel on Climate Change, it is stated that the climate crisis will grow even more if the temperature increase reaches 1.5 ^oC compared to the pre-industrial period (IPCC, 2020). Climate change is among the reasons for the emergence of zoonotic diseases, which have emerged especially after the 20th century and are likely to emerge in the coming years, threatening the whole world and turning into a pandemic. Curseu *et al.* (2009) stated that climate change and globalization can encourage the spread of avian flu and become a pandemic, and that global warming can change the migration patterns (routes) of birds as, consequently, the interaction between infected animals and humans. Wank and Crameri (2004) emphasized that climate change, habitat destruction, and modernization of agricultural practices, especially in developing countries, are the driving forces in the emergence of zoonotic diseases. These changes affect the interaction between pathogens and their hosts, between hosts and wild animals, and between livestock and people (Wank and Crameri, 2004). For instance; the reason for the higher levels of the spread of Tick-borne Encephalitis (TBE) virus in the Czech Republic has been linked to the effect of increased temperatures on the distribution of bird hosts from which the ticks were carrying this virus spread. The harsh winters due to climate change limit the movements of migratory ducks and cause them to gather in smaller areas, creating favorable conditions for H5N1 transmission in Europe (Fuller *et al.,* 2012). In addition to that, climate change may lead to the expansion of wetlands in eastern North America in parallel with the increase in precipitation and the congregation of more migratory ducks, which are the source of influenza (Fuller *et al.,* 2012). The emergence of West Nile Virus Outbreak, Rift Valey Fever, Dengue Fever, and O: 139 Cholera strain in new geographic regions is linked to El Nino release (Sachan and Singh, 2010). When biodiversity changes alter pathogen transmission dynamics, it is likely to spread to humans. The continuous emergence of Lyme disease in North America has been attributed to the decline of the red fox population, which has led to the abundance of small mammalian hosts of the pathogen (Baudron and Liégeois, 2020). In parts of Ghana, the eradication of lions and leopards has resulted in an increased abundance of baboons that come into contact with people at high risk of intestinal parasites (Baudron and Liégeois, 2020).

The Effect of Pandemic on Food Consumption

Food safety is defined as the state that all people have physical, social, and economic access to adequate, safe, and nutritious foods that meet their food needs and food preferences for a healthy life (FAO, 2009). The right to adequate food materialize when each individual, alone or with others, has physical, and economic access at all times to adequate food and means of supply (FAO, 2019a). The impact of pandemics on the change in food consumption habits, food safety, and the right to food has become more visible with the Covid-19 outbreak. In particular, the fact that people have to stay in their homes has changed their consumption habits and increased their anxiety about accessing healthy food (Janssen *et al*., 2021). During this pandemic, people started to take a stand against industrial agricultural products and foods. They seek ways to reach natural food, and the forgotten meals and foods inherited from the old generations have come to light again. During this period, consumers concerns about access to sufficient food and their demand for organic foods, which they consider safer in terms of nutrition and health, are increased. According to the Organic Product Performance Report published by the Organic Production Network (OPN) for the first quarter of 2020, it has been reported that consumers' buying behavior has changed in an unprecedented way with the impact of Covid-19 in the United States, and the dollar performance obtained from total organic products in March increased 22% compared to March last year and the dollar increase in January and February of this year was over 1.8% (Lutz and Long, 2020).

Taking animal protein every day regularly is vital for the antibodies, one of the body's defense mechanisms, to fulfill their functions and thus strengthen immunity. During the pandemic process, nutritionists emphasized the importance of the consumption of good quality animal products and the need to take protein regularly. It is accepted that the beneficial microorganisms found in animal products such as yogurt and kefir strengthen the immune system, the presence of antimicrobial properties of propolis, a bee product, and meat and dairy products obtained from naturally fed animals (pasture) are healthier and more nutritious. This idea has increased the demand for these kind of products (Kayan, 2021). Consumers' demand for organic certified chickens and eggs has increased in recent years due to the news about poultry being fed with genetically modified (GM) feeds. The continuity of these changes in diets will be inevitable, as diseases such as epidemics, cancer, abnormalities in the womb continue to increase with each passing year. More than 820 million people, in other words, one in nine people struggle with hunger, while more than 790 million people are estimated to be obese worldwide (FAO, 2019b).

While the pandemic process continues, the EU Commission's "From Farm to Fork" and "Biodiversity Strategy" reports were published in May 2020, which aim to fix food systems and turn them into fair, healthy, and environmentally friendly. Food systems cannot be resistant to crises such as the Covid-19 outbreak if they are not sustainable, according to the report. According to the decision of the Commission, pesticide use in EU countries will be reduced by 50% until 2030 and 25% of EU agricultural land will be allocated for ecological production (EC, 2020). In the United Nations Agroecology and Right to Food report, industrial production is stated to be largely dependent on fossil fuels and therefore not sustainable whereas ecological production is emphasized to be important to understand how nature works, to use the complementarity of plants and animals, to ensure a sustainable food production by protecting the food right of future generations, and to contribute to rural development (Schutter De, 2014).

First of all, the decision of countries to restrict foreign trade in some product groups with the coronavirus epidemic has further increased the importance of meeting food needs domestically. The disruptions caused by the Covid-19 crisis have exposed many of the vulnerabilities of today's food systems. Restrictions and market closures imposed in this process have led to workers' vulnerability across the food system, with access to safe and nutritious food at affordable prices. Those dealing with high-value, labor-intensive and perishable products (fruit, vegetables, fish, meat, and dairy products) required for good nutrition were disproportionately affected by this process. The Covid-19 outbreak has also highlighted the vulnerabilities in countries dependent on imports of food and agricultural inputs, creating a new perspective for shorter value and supply chain creation to increase market flexibility and reduce producer losses. Today's food systems fall short of the 2030 Agenda for Sustainable Development. While poverty and hunger are settled, obesity and related health problems and economic costs are constantly increasing. Food systems contribute significantly to increasing greenhouse gas emissions and constitute an important driving force for biodiversity loss. On the other hand, it is significantly affected by climate change (FAO, 2020a). During the pandemic period, countries have made intensive efforts to prevent a possible food crisis. Local administrations either gave the vacant lands they had to the producers or planted them with their own means for the needy. After this period, it is clear that issues such as food safety, sustainable agriculture, biodiversity, and access to healthy food will often come to the fore due to such epidemics. Because of this reason, an ecological transformation will be inevitable in both crop and animal production. According to the report named Guiding The Transition To Sustainable Food And Agricultural Systems of the United Nations Food and Agriculture Organization (FAO), agroecology; simultaneously applied to food design and management and agricultural systems; It is an approach integrated with ecological and social concepts and principles. In addition to this, taking into account the social situation needed for a sustainable and fair food system; It is a system that seeks to optimize the interaction between plants, animals, humans, and the environment (FAO, 2018).

The Effect of Pandemic on Livestock Sector

In this epidemic period, the governments' taking measures such as travel, trade, and curfew caused some difficulties in the supply of agricultural inputs and especially in the marketing of products with short durability, such as animal products**.** The increase in the prices of imported agricultural inputs (fertilizer, medicine, feed, etc.) due to the import-export imbalances that occurred during the pandemic period and the increase in exchange rates caused the farmers engaged in both herbal and animal production to enter the bottleneck. China is the most important producer and consumer of phosphate, sulfur, and sulfuric acid (Seleiman *et al.,* 2020). The Covid-19 outbreak that occurred in China affected the fertilizer industry by disrupting both the fertilizer and fertilizer raw material movement. For some countries that are synthetic fertilizer importers, producers have been advised to add biological and organic fertilizers in addition to synthetic fertilizers (Seleiman *et al.,* 2020). In the production of forage plants, which are one of the main inputs of animal husbandry, both raw materials and synthetic fertilizers, medicines, et cetera used in production. Countries that import inputs have been the countries most affected by the pandemic process. It is a critical decision for the livestock sector that America and Brazil, which are the most important soy and corn exporters due to Covid-19, want to use these products for domestic consumption instead of exporting in large quantities (Seleiman *et al.,* 2020). Supply chain disruption due to the pandemic further delayed the feed supply. Again, disruption of the global trade flow due to restrictions in Brazil, the world's largest soy exporter, has reduced soy supply to feed mills. For these reasons, the increase in input prices will cause an increase in the prices of animal products. Import restrictions will greatly affect Africa and developing countries that require imported inputs to sustain production, or whose meat and milk consumption is dependent on imports. For instance; In Iran, since 80% of the feed inputs of the poultry farms are dependent on imports and because of the sanctions that the enterprises cannot access corn and soybean pulp, the chicks have been killed. Similarly, in countries where access to animal markets, slaughterhouses, and markets is restricted, producers will be exposed to higher production costs and product loss because they have to keep their livestock or shed their milk (FAO, 2020b).

During the pandemic process, countries whose economy depends on the livestock sector had significant economic losses regarding food safety. Likewise, while the increase in avian influenza incidence in 2005-2006 decreased the demand for poultry, it directed consumers to other livestock products. It is emphasized that the Covid-19 period and other pandemics that will emerge from now on may adversely affect animal production and those whose livelihood is animal production. The impact of the livestock supply chain from the pandemic process may limit access to the market, especially by nomadic livestock producers (FAO, 2020c). Many livestock markets have been closed at the west of Africa, bovine and ovine prices have dropped by more than half, and this situation has forced migrant livestock breeders to quit animal husbandry en masse (FAO, 2020b). Disruptions in the tourism sector due to closures of restaurants, other places of mass consumption, and travel restrictions are likely to adversely affect the supply chain of animal products (Seleiman *et al.,* 2020; UNCTAD, 2020).

Ecological Animal Husbandry

Organic animal husbandry is a system that encourages the use of organic and biodegradable inputs in the ecosystem in animal feeding, animal health, animal housing, and reproductive systems (Chander *et al.,* 2011).

The use of too many chemicals in agricultural research caused some problems in plant and animal breeding with the rise of the petrochemical industry in the early 1900s. Agrochemicals, veterinary medicines, antibiotics, and improved feeds can reduce production costs and increase food supply in various livestock systems around the world. However, nowadays, quality-conscious consumers are looking for healthy foods that are environmentally safe and do not leave chemical residues, together with product traceability and produced via organic methods including high animal welfare. Livestock has always played a key role in organic production systems. Between 1920 and 1950, the typical organic farms of Great Britain, Europe, and North America combined the breeding of livestock, forage crops, and food production. This system met all the needs of a farmer, while the waste obtained from livestock was used as fertilizer in crop production, crop production residues were used as feed in animal husbandry (Wolde and Tamir, 2016).

Businesses in organic agriculture are based on a self-feeding and closed production system that enables animal, plant, human, and food interaction (Figure 1). Organic agriculture aims to ensure sustainability in agriculture by protecting the health of humans and other living creatures to the maximum without polluting the environment, soil, water resources, air, and agricultural products. Among the main principles of organic agriculture in the production for this purpose are production in harmony with nature, appropriate crop rotation application, and the application of a closed system agriculture model. The closed system agriculture model is defined as a type of agriculture in which the resources of the enterprise are used and the enterprise can be as self-sufficient as possible. Especially in this

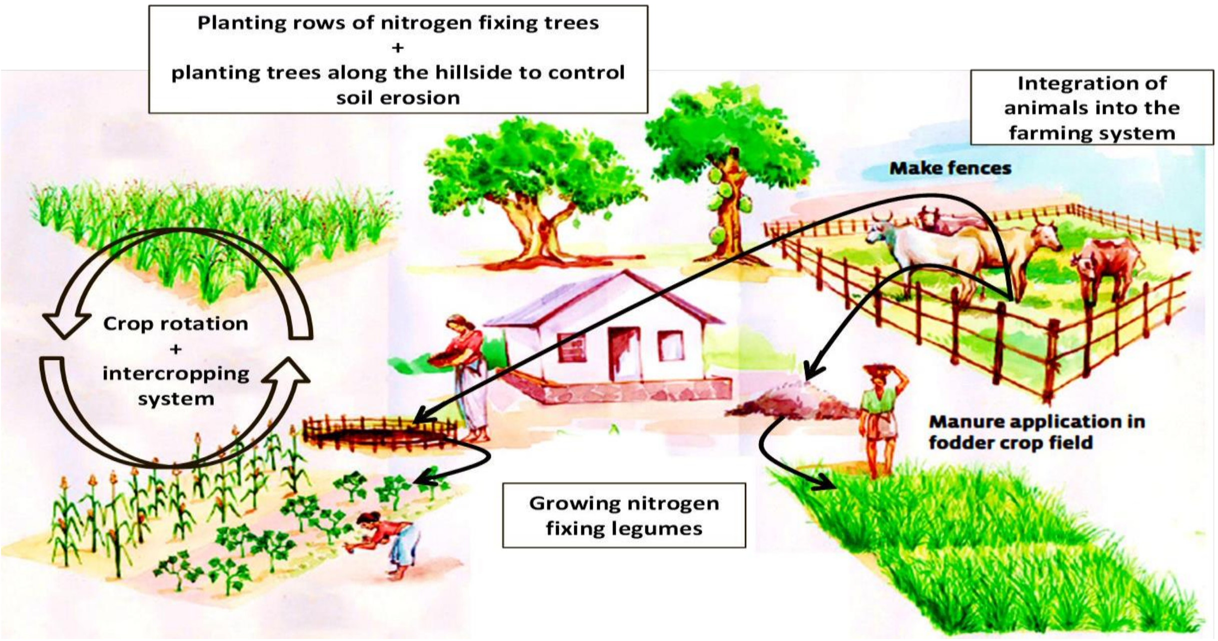

Figure 1: Some Organic Farming Methods (FAO, 2015).

system where plant production and animal husbandry are combined, it is important to evaluate the products produced in the enterprise as animal feed and to use animal wastes obtained from animals as organic fertilizers in plant production, in terms of providing the cycle in production (Duman *et al.,* 2009). Organic or ecological animal husbandry is a closed system production model that takes care of human health, animal health and welfare, encourages alternative livestock production systems, such as silvapastoral systems that less encourage outbreaks. It is also a model that is environmentally friendly, compatible with nature, chemically synthesized veterinary medicinal products, antibiotics and inputs obtained from genetically modified organisms are not used, all kinds of waste are evaluated as re-inputs and inputs are within the enterprise as much as possible (Altieri and Nicholls, 2020). Animal manure, which is seen as waste in most conventional livestock enterprises, contributes to the formation of a new product in organic livestock enterprises. Organic livestock farming is pasture-based and still does not allow antibiotics used as feed additives in some countries. In a study conducted to compare organic and traditional raw milk quality in the Netherlands, the conjugated linoleic acid (CLA) and omega 3 fatty acids levels of organic milk were found to be significantly higher (Bloksma *et al.,* 2008). Butler *et al.* (2008) compared the difference between fatty acid and antioxidant profiles of milk from traditional and organic systems in England and Denmark, and found that milk from organic farms had higher concentrations of nutritionally desirable fatty acids, conjugated linoleic acid, α-linolenic, α-tocopherol, and carotenoids. In addition, the impact of reproduction technologies such as superovulation, Multiple Ovulation and Embryo Transfer (MOET) or OPU-IVP-ET on welfare and integrity has already been discussed. It is accepted that reproduction techniques should be natural (Nauta *et al.,* 2001). According to the International Federation of Organic Agriculture Movements (IFOAM) standards, while artificial insemination is allowed, embryo transfer, genetic manipulation and hormonal synchronisation are not permitted (FAO, 2021).

According to the joint data of Research Institute of Organic Agriculture (FIBL) and International Federation of Organic Agriculture Movements (IFOAM), organic agriculture activities carried out with 2.8 million producers on 71.5 million hectares of land in 186 countries in 2018 reached 96.7 million Euros in the global market. The countries with the largest organic market in 2018 are the USA (40.6 billion Euros), Germany (10.9 billion Euros), and France (9.1 billion Euros). The highest amount of money spent on organic products in 2018 was in Denmark and Switzerland with 312 Euros per capita, and later in Sweden with 231 Euros (FIBL and IFOAM, 2020).

In Australia, 97% of the 35.69 million hectare organic production areas, which continues to grow its organic lands in 2018, are large pastures used for feeding cattle. In terms of organic production, Australia is followed by Argentina with 3.63 million hectares and China with 3.14 million hectares. Organic pasture areas worldwide increased by 2.9% in 2018, accounting for more than two-thirds (48.2 million hectares) of the total organic farmland. Current statistics show that organic pastures constitute 34% of organic agricultural lands, 27% of organic arable lands, and 18% of organic permanent products. The organic livestock sector is developing rapidly in European countries. Organic livestock farming in many countries started with beef, lamb, and milk production. According to 2018 data, 4.85 million cattle, 5.9 million sheep, 1.4 million pigs, and 56.5 million poultry are organically grown in Europe. Between 2009 and 2018, the biggest increase (128%) was in poultry due to high egg demand. Growth in organic livestock in this decade was 88% in beef and dairy cattle, 69% in sheep, and 105% in pigs (FIBL and IFOAM, 2020). Top three in organic cattle breeding are Germany (771.320), France (751.382), and Austria (421.324), while Greece (1.299.677), France (1.132.809), and the United Kingdom (826.598) are in organic sheep farming and Germany (178.200), Denmark (488.886) and France (317.925) are in organic pig farming. Organic cow milk production in EU countries has almost doubled since 2007 to meet the growing demand for milk and dairy products. According to the 2018 data, there are 5.113 cattle, 1.25 million poultry, 10.475 sheep-goats, and 51.272 beehives in Turkey (FIBL and IFOAM, 2020).

According to USDA PSD data, the use of corn and soy for feed purposes in conventional livestock breeding was 36-38% and 20-21% in the USA between 2016/17 and 2018/19, while the use of organic corn and soy was 52-62% and 77-86%, respectively (MERCARIS, 2020). The share of all animals in organic production remains inadequate compared to some crop production groups due to the insufficient organic feed obtained from local sources and the difficulties in obtaining traceable certified feed imports (FIBL and IFOAM, 2020).

The Importance of Ecological Animal Husbandry in Pandemics

The experienced process in the Covid-19 pandemic has revealed how closely linked human, animal, and ecological health is (Altieri and Nicholls, 2020). Human behavioral changes are caused by population growth, economic and technological development, and associated agricultural expansion; It creates new and more intense interactions between humans, animals, and wildlife. While meeting the food requirements of the increasing global population, there is a need for sustainable agricultural food systems that protect human health, biodiversity, and the environment and minimize the risk of emerging diseases (Jones *et al.,* 2013).

Many countries engaged in industrial animal husbandry import genetically modified soy or soy feed for animal feeding purposes. While herbicides used in industrial soybean production enter the digestive systems of animals, causing a decrease in beneficial bacteria (Jespersen, 2017), this may cause animals to be exposed to more diseases. In a study that compared chickens fed with traditional and the organic feed, it was found that although the chickens fed with organic feed came from behind in weight gain, caught chickens fed with traditional feed, and chickens fed with organic feed showed an improved immune reactivity (Huber, 2012). Organic livestock farming's not allowing the use of antibiotics is important for both human and animal health. According to the WHO, the use of antibiotics in livestock reduces the effectiveness of antibiotic treatment applied to humans (WHO, 2015). According to the European Medicines Agency's Sales of veterinary antimicrobial agents in 30 European countries 2016 report, for 1 kg of biomass (mg / PCU), the countries that used the most veterinary antimicrobial agents from 30 European countries between 2010 and 2016 were Cyprus (453.4 mg), Spain (362.5 mg) and Italy (294.8 mg) (EMA, 2016). In industrial livestock, antimicrobials are often used for growth promotion, disease prevention, and treatment, which promote the evolution of antimicrobial resistance in zoonotic pathogens (FAO, 2019a). The fact that organic animal husbandry promotes biodiversity and allows animals to roam freely provides low concentrations of zoonotic pathogens in these herds. While avian flu was of high intensity in conventional farms, it has been shown that there was low mortality and difficulty in spreading in small farms called backyard poultry and in flocks with diversity among poultry (GRAIN, 2006).

Organic animal husbandry demands for animals be provided with living conditions and opportunities suitable for their physiology, natural behavior, and well-being (Vaarst and Alrøe, 2012). In organic livestock farming, the movement of animals in wider areas and the low density of animals falling in a certain area prevents the animals from being stressed and reduces the risk of diseases. Animal welfare has also become a marketing argument for organic produce, and in some countries, consumers think that organic farming products as more 'animal friendly' than traditional products (Lund and Algers, 2003).

It is also important for countries' food safety that organic systems are self-feeding systems. Because organic livestock farming is either slightly dependent or not dependent on feed crops (soy, corn and etc.) used in industrial animal husbandry and imported by many countries. Controlled grazing of animals on the pasture not only allows pastures to be maintained and regenerated but also maximizes soil fertility, animal welfare, and quality animal food production (Escribano, 2018). Due to foreign trade restrictions, especially during the Covid-19 pandemic, countries and international organizations have started to focus more on food safety, self-sufficiency, and sustainable food production systems. These issues will be constantly on the agenda during and after this process (Gülçubuk, 2020).

In recent years, especially in parallel with the increase in environmental and health awareness, the demand for organic products has increased in EU countries, the USA, and other developed countries. In a study conducted in the USA, it was investigated why consumers prefer organic products. According to this study, 66% of consumers think that organic products are healthy and more nutritious, 38% taste better, 26% have positive effects on the environment, and 30% think that there is no hormone or drug residue in terms of food safety (Durak Kılıçaslan, 2015).

According to a report prepared in the UK, the coronavirus pandemic has increased the demand for organic and sustainable foods. According to the report, there was a 25-30% increase in the sales of online natural food retailers during the pandemic process (Ecovia Intelligence, 2020) . While existing customers from physical retailers were shopping more, so did customers consuming new organic products. During the Covid-19 period, consumers' awareness of nutrition and health increased and they bought more organic food to strengthen their immunity. The report states that the demand for organic foods will continue after the fear of the pandemic has passed. For example, the BSE crisis in 2000 increased the demand for organic meat products in Europe and sales remained high in the following years. Similarly, emerging SARS in China (and Asia) led to an increase in demand for organic food. The melamine scandal in 2008 increased the demand for organic baby food in China, and within a few years, the Chinese organic baby food market has become the largest in the world. Organic foods were first introduced on a large scale in the early 1990s, and global organic product sales exceeded USD 50 billion in 2008 and USD 100 billion in 2018. As the Covid-19 pandemic changes the way we shop and eat, the organic food market is estimated to exceed USD 150 billion in the next 5 years (Ecovia Intelligence, 2020).

The Covid-19 pandemic has led to the need to focus on many current issues such as antimicrobial resistance, zoonotic diseases, climate change, food fraud, and the digitalization of food systems, each of which has potentially significant consequences for food security. In the same way, the importance of protecting environmental resources and biodiversity as a natural buffer against diseases has shed light on the significant level of habitat degradation associated with land-use changes (FAO, 2020a). Ecological animal husbandry will become more important and necessary in the future in terms of solving problems such as human health, animal health and welfare, environment, climate, biodiversity, food security of generations, and food safety in general.

Conclusion

It is clear that pandemics that existed in the past and today will be inevitable in the future. The destruction of natural habitats, industrial agriculture, industrialization, and the decrease in biodiversity disrupt the ecological balance and create an environment for pandemic formation, endangering both human and animal health. This period revealed the importance of agricultural production, especially ecological production, and the desire of people living in cities to purchase agricultural products without intermediaries from producers, support small family businesses and settle in rural areas and produce their own food increased. In other words, the awareness of consumers about food has increased. With projects such as "Waste-Free Kitchen" and "Save Food, Protect Your Dining Table", the consumers have become more conscious.

Ecological animal husbandry transformation is a solution proposal to these problems that are likely to exist in the future and even increase their severity. It is clear that ecological animal husbandry is important in terms of both human and animal health and welfare to prevent pandemics by reducing the pressure on the ecological balance, and its importance will be understood more in the next 20 years. In countries that are dependent on imported inputs in animal production, the Covid-19 period has shown that domestic production is necessary for food safety, industrial feed production will not be sustainable for animal feeding, there is need to improve pastures with agroecological models, and a closed agricultural system model that is not dependent on external sources is important.

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Author contributions

All authors contributed equally to the study.

Conflict of Interest

The author declare no conflicts of interest.

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LIVESTOCK STUDIES

RESEARCH PAPER

The Effect of Different Management Systems and Racing on the Stress Level of Arabian Horses

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Abstract

Considering the stud farms and hippodromes, there are differences between them both in terms of management systems. The study population consisted of 40 Arabian mares. The breeding systems of horses at the stud farm and hippodrome, the physical dimensions of their boxes, agonistic behaviors, stereotypic behaviors, and intra/inter-species interactions were examined and evaluated comparatively by considering their physiological stress and behavioral responses. The salivary cortisol analysis was performed at rest on the horses at the stud farm and pre-post race on the horses at the Hippodrome. Statistical difference was not found between the stereotypic and agonistic behaviors of the horses but the longer-term effects of the absence of intraspecies interaction should be investigated. There was no statistical difference in the resting horse's saliva cortisol value housed at the stud farm (0,84 ng/ml) and hippodrome (0,52 ng/ml). It was determined that the pre-race cortisol value (0,52 ng/ml) was significantly lower than the post-race (3,82 ng/ml) value. In order for the horses to have a long and healthy sports life, the welfare of the horses should be structured by considering the training, behavior, and management systems with a holistic approach, and intra-species interactions should not be overlooked.

Introduction

Stress is defined as a response to environmental stimuli that threaten an organism's homeostasis (Ramos and Mormède, 1998). The decrease in welfare also causes stress in the horses, which may cause the horses to not be able to benefit from the desired efficiency as a result of stereotypical behaviors. However, as a result of the increase in the frequency of agonistic behaviors, accidents and injuries may occur. Long-term exposure to stressors or short-term exposure to high-intensity stressors can lead to reduced animal welfare. In the case of chronic stress, it can cause some health problems. The organism of the animal under stress cannot renew its biological resources, so this situation affects the animal in a negative way (Moberg and Mench, 2000; Etim et al., 2013). Minimizing stress in horses used for riding is necessary for animal health and welfare (Jung et al., 2019). Because competition is a mix of various stress factors, simply being in the competition arena before the competition can cause a classic physiological stress response in horses that can affect racing performance. Healthy sport horses recover rapidly at the end of the competition season when the stimuli that cause stress have gone (Negroa et al., 2018).

Horses are herd animals in their natural life; they do not live alone (Klingel, H., 1967). However horses need daily interaction with intraspecifics to lead a normal life and for their mental health (Landsberg, 2013). Although housing the horses in individual boxes limits their natural behavior (especially locomotor and social behavior), this housing system is widely preferred, especially for racehorses used for flat racing. Although the free presence of horses in paddocks and pastures has a positive effect on animal welfare, this system is not preferred by many horse owners because it causes sports horses to get injured (Houpt, 2005; McGreevy et al., 1995; Christensen et al., 2002; Houpt and McDonnell, 1993). However, the presence of paddocks where horses can socialize with other horses and show their natural behavior is critical for horse welfare. Domestic horses in a herd interact socially with other horses in their paddocks and get the opportunity to exercise by moving throughout the day (Erber et al., 2013). In socially organized animals, the absence of their conspecifics causes stress responses (Schmidt et al., 2010). Although daily riding or training reduces the need for additional physical activity in horses (Alexander and Irvine, 1998), it cannot fully meet the need for free exercise (Werhahn et al., 2011).

A positive horse-human relationship is a key factor in establishing reliable interaction with animals (Hausberger et al., 2008). When judged on the basis of animal welfare, the familiar (friendly) response of horses to humans is the most desirable behavior for establishing a safe human-horse relationship. Despite the long history of horse-human interaction, both professional and amateur people who come into contact with horses are exposed to horse-related accidents (Mills et al., 2000). Studies show that there are problems related to horse-human interaction in administrative areas such as care, management, and training (Hausberger et al., 2008). Undesirable horse behaviors (agonistic and stereotypic behaviors) are considered inappropriate responses to the current situation and may pose a danger to both animals and humans (Cooper and Albentosa, 2005). However, they may also display normal but undesirable behaviors in response to certain environmental deficiencies (Mills et al., 2000). These are agonistic behaviors such as rearing, kicking, and biting (Hausberger et al., 2008). Stereotypical or agonistic behaviors play an important role as an indicator of poor welfare and chronic stress (Cooper and Albentosa, 2005; Nicol, 1999; Mason and Latham, 2004). Stereotypical behaviors are defined as repetitive, unvarying, and nonfunctional behavior patterns (Mason, 1991). The most important way to ensure a horse-human dyad for the welfare of both horses and humans is to create and develop a strong positive interaction (Hausberger et al., 2008).

The relationship between stress and the animal's metabolic system has been demonstrated by many studies (Minton, 1994; Salak-Johnson and McGlone, 2007), and different methods have been developed to assess stress levels (Valera et al., 2012). Most of the techniques used to measure stress in animals include invasive procedures such as blood sampling that can induce a stress response (Stewart et al., 2005). One of these techniques is the measurement of cortisol in saliva, which has been highlighted as a more useful method than plasma or urine cortisol for stress assessment in horses (Schmidt et al., 2009; Bohák et al., 2013; Cordero et al., 2012). Cortisol in the saliva is a direct reflection of free cortisol concentration. It is a much more sensitive indicator of blood and adrenocortical activity than plasma "total" concentrations. Since cortisol is rapidly diffused into saliva, salivary cortisol concentrations reliably reflect blood cortisol concentrations (Peeters et al., 2011; Schaefer et al., 2002). Changes in cortisol hormone levels in horses can be symptoms of both acute and chronic stress (Hada et al., 2001; Nunez et al., 2014). An animal's response to negative stimuli includes the release of cortisol and the immediate response of the sympathetic-adrenomedullary system. During short-term stress, cortisol can increase vitality with energy mobilization (Raynaert et al., 1976) and cause changes in behavior (Korte et al., 1993). However, cortisol is also released when the stressor persists, emphasizing the presence of chronic stress (Schaefer et al., 2002). These chronic stress symptoms may include reproductive problems, ulcers, an increased incidence of disease, decreased body weight, and the development of abnormal behaviors such as stereotypes (Mills and Nankervis, 1999).

Evaluation of factors affecting animal welfare has been the basis of many scientific studies in recent years. Most of them focus on a specific potential impact factor (e.g., management; Casamassima et al., 2001; Meunier-Salaün et al., 1987; social behaviors; Grignard et al., 2000; Van Reenen et al., 2000; Bouissou et al., 2001; Wechsler et al., 1997; feeding; Freire et al., 2009). However, only a multifactorial approach with maximum management parameters would be beneficial in terms of an overall assessment and thus an improvement of the actual results on animal welfare.

Arabian horses are bred in stud farms where they live together with their conspecifics until they are 3 years old, and then they are taken to hippodromes where they are socially isolated from their conspecifics for flat races. The aim of this research is to examine the housing conditions, stereotypic behaviors, agonistic behaviors, and intra- and inter-species interactions of horses used for breeding and flat racing with a multifunctional approach and to use salivary cortisol concentration as a physiological stress parameter in determining the stress caused by race in flat races. As a result of the research, the welfare of Arabian horses in hippodrome and farm conditions was evaluated by considering physiological and behavioral stress parameters, and suggestions were made to increase welfare. At the same time, by determining the stress levels of horses used in different fields (for breeding and racing) and horses competing in hippodromes during the racing season and out of season, problems and solution suggestions were put forward from a scientific perspective.

Materials and Methods

This study was carried out within the scope of the permission of Eskişehir Osmangazi University Animal Experiments Local Ethics Committee 17/02/2021 date, 839/2021 number of decision.

Experimental Animals

Within the scope of the research, a total of 40 Arabian mares were used, 20 of which were breeding at a stud farm in Mahmudiye and 20 of which were in the hippodrome for flat racing. Both groups were housed using the boxing system, but while the horses had access to the paddock at the stud farm, there was no paddock at the Hippodrome. Horses at the stud farm were in the paddock for an average of 12 hours per day during the breeding season. The mares at the stud farm kept in social contact with the other mares and foals in the paddock. At the same time, they could see each other in their box in the stable. The mares in the hippodrome could not see other horses in their box in the stable.

Wheat straw was used as litter material in the boxes at the stud farm and wood shavings in the hippodrome. While the horses at the stud farm always had access to water, they were watered by hand at the hippodrome. Horses in both groups were fed meadow grass and alfalfa hay as roughage and barley paste as concentrate feed. Horses in the stud farm consumed an average of 5-6 kg of feed per day, and horses in the hippodrome consumed 6-8 kg of concentrate feed in two parts a day, in the morning and in the evening. Meadow grass was given ad libitum to both groups of horses. Horses at the stud farm were given 4 kg of alfalfa grass daily in the paddock between 10:00 and 17:00. The horses in the hippodrome were given 1 kg of alfalfa grass after training.

Experimental Design

Stereotypical behaviors, agonistic behaviors, intra-species and inter-species interactions of the horses, and physical parameters of the stable were recorded with the help of observations and information received from the researchers. The agonistic behavior of horses has been studied on the basis of horse-human interaction. In addition, pre- and post-race salivary cortisol levels were compared in order to determine the stress on Arabian horses from flat races.

A total of 20 Arabian mares were bred in the stud farm and hippodrome (10 horses were housed in the stud farm and 10 horses in the hippodrome); saliva samples were taken twice for the horses in the hippodrome, in the racing season and out of the racing season, and once for the mares at the stud farm, in the resting time, between 9:00 and 11:00 in the morning. The salivary cortisol concentrations in the samples taken were analyzed and compared with the other data in the study. Other data (stereotypic behaviors, agonistic behaviors, intra-species and inter-species interactions, physical parameters of the stable) were obtained from all horses in the study (40 Arabian mares).

Within the scope of the research, the physical parameters of the stable in the stud farm and hippodrome were recorded by observing them with the help of "Table 1: Physical Parameters" were presented below, and obtaining information from the researchers.

The “Stereotypic Behaviors of Horses” presented in Table 2 within the scope of the research were created in the light of the data revealed in the research of Mills (2005) and Lesimple and Hausberger (2014). The stereotypical behavior of the horses was recorded with the information and observations taken from the researchers.

By the researcher's observations, the agonistic behavior of the horses was determined when the groom entered the box and the horse was being led and as presented in Table 3 "Agonistic Behaviors of Horses". Within the scope of the research, the presence of each agonistic behavior in the "Agonistic Behavior of the Horses" table was scored with “1”. Agonistic behaviors of horses are scored between 0-17 within the scope of the table.

Within the scope of intraspecies interactions between horses and humans, their socialization status and interaction duration with other horses were evaluated with the help of the "Intraspecies and interspecies interaction table" presented in Table 4. Socialization and interaction durations with humans were determined within the scope of interaction between species.

Table 1. Physical Parameters of the Stable

Physical Parameters	Measurement
Box Dimensions	(width x length x height)
Window Dimensions	(width x length x height)
Height of boxing window from ground	meter
The time the boxing window stays open during the day	hour a day
Height of boxing door window from ground	meter
The time the door window stays open during the day	hour a day
Paddock area per horse	m^2

Table 2. Stereotypic Behaviors of Horses (Mills, 2005; Lesimple ve Hausberger, 2014)

Stereotypic Behavior	Description	Incidence Frequency (day/week)
Wind sucking/Wood chewing	The horse grasps a fixed object with its incisors, pulls backward and draws air into its esophagus	
Weaving	Obvious lateral movement of head, neck, forequarters, and sometimes hindquarters	
Striking with forelimb	The horse hits the door or wall with one of its forelegs	
Head tossing/nodding	Vertical movements of head and neck,	
Box walking	Repetitive tracing a route within the stable	

Cortisol analysis from saliva is a non-invasive method. The cortisol test was performed with the Roche Cobas device by ELISA method. Salivette Tube (Salivette Cortisol; Sarstedt, Nümbrecht-Rommelsdorf, Germany) was used to collect saliva. The roll of cotton was removed from the inner tube, and it was brought to a saturated state by moving it between the teeth and cheek of the horse for about 2 minutes. After the roll of cotton was placed back into the inner tube, the tube was closed. The Salivatte instrument was centrifuged at 1000 g for 2 minutes to separate the saliva from the inner tube and exit to the outer tube.

The SPSS 25 package program was used in the analysis of the data. After determining whether the data were suitable or not for normal distribution, the Wilcoxon Signed Rank Test was used to determine whether there was a significant difference between the variables.

Results

When the agonistic behaviors of the horses housed in the stud farm were evaluated, it was seen that the score (the score value of each behavior was "1" within the scope of the research) was in the range of 0-5. When the distribution of agonistic behaviors was examined, it was seen in Table 5. Agonistic Behaviour of Horses in Stud Farms/Hippodromes; 0 at the rate of 17.64%, 1 at the rate of 11.76%, 2 at the rate of 11.76%, 3 at the rate of 11.76%, and 5 at the rate of 5.88%. When the agonistic behaviors of the horses housed in the hippodrome were evaluated, it was seen that the score was in the range of 0-6. When the distribution of agonistic behaviors was examined, it was seen that it was 0 at the rate of 17.64%, 1 at the rate of 11.76%, 2 at the rate of 11.76%, 3 at the rate of 11.76%, and 5 at the rate of 5.88%. While stereotypic

Table 3. Agonistic Behavior of the Horses.

Horse-horse interaction (McDonnell, 2003)	Horse-human interaction (McGreevy et al., 2009)
Alert	Staring at horse while standing within its visual field
Ears laid back/pinned	Ear threat towards human
Avoidance/retreat	Horse avoiding being caught
Balk	Horse ceasing forward movement while being led
Bite threat	Horse threatening to bite handler
Rearing	Rearing towards handler
Chase	Horse chasing human out of the stable, paddock or roundpen
Head bump	Head-to-head contact with human
Head on neck, back or rump	Head-to-neck, back or rump contact with human
Head-bowing	Horse bowing towards handler
Herding and driving	Horse causing human to move in one direction
Kick	Horse kicking handler
Kick threat	Horse threatening to kick handler
Nip	Horse nipping handler
Parallel prance	Prancing alongside handler
Push	Barging
Stomp	Stomping at handler

Table 4. Intraspecies and interspecies interaction table.

Intra/interspecies interaction	Stud Farm Duration	Hippodrome Duration
Duration of horses in the paddock	12 hour/day	0 hour/day
Frequency of horses in the paddock	7 day/week	0 day/week
Duration of see other horses	24 hour/day	3 hour/day
Duration of hear other horses	24 hour/day	24 hour/day
Socialization time of the horse with other horses	12 hour/day	3 hour/day (during training)
Frequency of socialization of horse with humans	7 day/week	7 day/week
Socialization time of the horse with humans	0,5 hour/day	4 hour/day

Table 5. Agonistic Behaviour of Horses in Stud Farm/Hippodrome

Horses in Studfarm	Agonistic behaviour(%)	Horses in Hippodrome	Agonistic behaviour(%)
S1	5.88	H1	11.76
S2	29.4	H2	0
S3	0	H3	17.64
S4	0	H4	17.64
S5	5.88	H5	5.88
S6	11.76	H6	0
S7	11.76	H7	5.88
S8	17.64	H8	0
S9	0	H9	11.76
S10	17. 64	H10	35.29

behavior was not observed in the horses housed in the stud farm; one stereotypic behavior was observed in 11.76% of the horses housed in the hippodrome.

Inter-species interaction durations between groups; while the horses housed in the stud farm were 0,5 hour/day, and the horses housed in the hippodrome were determined as 4 hours/day. When the intra-species interaction times between the groups were evaluated, it was determined that the horses housed in the stud farm were 12 hours/day, and the horses housed in the hippodrome were 3 hours/day. While the horses housed in the stud farms could show their natural behavior as they were free in the paddock during interspecies interaction, the horses in the hippodrome stayed together with their conspecifics during training, riding, or exercises.

When the paddock area per horse between the groups was evaluated, while the horses housed in the stud farm have an area of 2250 m^2/horse, there was no paddock for the horses housed in the hippodrome. In both systems, the box size was 16 m^2. The window area of the boxes was 0.5 m^2 at the stud farm and 0.6 m^2 at the Hippodrome.

When the pre-race (at rest) and post-race cortisol values were compared in the hippodrome, it was determined that the pre-race cortisol value (0.52 ng/ml) was significantly lower than the post-race (3.82 ng/ml) value *($P<0.01$)*. At rest, the cortisol determined from the saliva of the horses housed in the stud farm was 0.84 ng/ml on average, while the average cortisol determined from the saliva of the horses housed in the hippodrome was 0.52 ng/ml. There was no statistical difference in the resting horses housed at the stud farm and hippodrome *($P> 0.05$)*.

Discussion

In Turkey, Arabian horses are bred by the government (Anatolian Agricultural Enterprise, Cifteler Stud Farm, Sultansuyu Stud Farm, Karacabey Stud Farm) or in private stud farms, and most of them are used for flat racing and breeding in stud farms. Considering the stud farms and hippodromes, it is seen that there are differences between them both in terms of the physical conditions and management systems of horses.

Horses are generally transferred from the places where they are housed in groups (stud farms) to the areas where they are housed individually (hippodromes) for their training and for flat races. Erber et al., (2013), based on physiological parameters, revealed that the restriction of living space in individual housing and keeping horses separate from other horses are potential sources of stress and that mares with access to the paddock have higher movement activities than those in individual boxing. However, it was stated that horses with access to the paddock showed less stress-related behavior than those without. Studies have shown that horses kept in social groups were easier to manage (Søndergaard and Ladewig, 2004) and required less time to reach a certain level of training

Table 6. Intra/interspecies Interaction

Intra/interspecies interaction	Stud Farm Duration	Hippodrome Duration
Duration of horses in the paddock	12 hour/day	0 hour/day
Frequency of horses in the paddock	7 day/week	0 day/week
Duration of see other horses	24 hour/day	3 hour/day
Duration of hear other horses	24 hour/day	24 hour/day
Socialization time of the horse with other horses	12 hour/day	3 hour/day (during training)
Frequency of socialization of horse with humans	7 day/week	7 day/week
Socialization time of the horse with humans	0,5 hour/day	4 hour/day

than horses housed individually (Rivera et al., 2002). In a study demonstrating the positive effect of the presence of their conspecifics on horses, it was stated that the stallions housed individually showed higher levels of aggression than the stallions housed in the herd (Christensen et al., 2002). Löcken et al. (2016) revealed in their research that after being kept alone in horseboxes for 6 months, a positive cognitive perception was formed in horses that were grazing for ten days and in contact with their conspecifics. As a result of the research, they mentioned the importance of social behaviors such as exploration, social interaction, play, and grooming for the welfare of horses. In another study examining the behavior and stress levels of horses with and without paddock access during training, they stated that the individual housing of the horses greatly restricted their natural behavior and placed stress on the horses throughout the day. For this reason, they drew attention to the importance of free exercise and social interaction in terms of horse welfare (Werhahn et al., 2012). Stereotypic behaviors in horses are considered an indicator of chronic stress rather than a coping mechanism (Broom, 1983). In the meantime, stress can adversely affect the welfare and health of animals. Hovey et al. (2021), revealed that there is a clear relationship between behavior and cortisol concentrations in horses in high-stress environments. In a study by Bachmann et al., (2003), it was suggested that wood-chewing horses were more sensitive to stress and less physiologically and psychologically adaptable than control horses. In a study by Löckener et al., (2016), it was found that stereotypic mares had a lower mating success than non-stereotypic mares. In Turkey, Arabian horses are bred by the state or in private stud farms, and most of them are used for competition on flat running and for breeding in stud farms. Considering the stud farms and hippodromes, it is seen that there are differences between them both in terms of the physical conditions and management systems. With the research, agonistic and stereotypic behaviors of horses were determined and examined in relation to stress. In this study, the effect of two different housing types (with/without paddock), on the welfare of horses was examined. Within the scope of the research, there was no statistical difference in the agonistic and stereotypic behaviors of the horses

Table 7. Pre-race/post-race cortisol value

Horses in Hippodrome	Pre-race Cortisol Value (ng/mL)	Post-race Cortisol Value (ng/mL)	P
H1	<0.5	6.40	
H2	<0.5	1.72	
H3	<0.5	5.31	
H4	0.70	2.49	
H5	<0.5	9.54	
H6	<0.5	3.55	
H7	<0.5	3.81	
H8	<0.5	1.85	
H9	<0.5	3.12	
H10	<0.5	0.40	**
Mean	**0.52±0.02**	**3.82±0.83**	

***: P<0,01*

Table 8. Cortisol Value at Rest in Stud farm/Hippodrome

Horses in Studfarm	Cortisol Value at Rest (ng/ml)	Horses in Hippodrome	Cortisol Value at Rest (ng/ml)	P
S1	2.88	H1	<0.5	
S2	0.99	H2	<0.5	
S3	0.5	H3	<0.5	
S4	0.99	H4	<0.7	
S5	0.5	H5	<0.5	
S6	0.63	H6	<0.5	
S7	0.5	H7	<0.5	
S8	0.5	H8	<0.5	
S9	0.5	H9	<0.5	
S10	0.5	H10	<0.5	-
Mean	**0.84±0.23**		**0.52±0.02**	

P>0.0

housed in the stud farm and hippodrome. The reason for this could be explained by the fact that the horses in the hippodrome within the scope of the research have been in the hippodrome for less than 6 months. While the percentage of stereotypical behavior in horses at the stud farm was 0, it was determined as 11.76% in the hippodrome. It is thought that the inability of horses to show their social behaviors in the long term would be increased the frequency of agonistic and stereotypic behaviors.

A study revealed that 216 veterinarians from Switzerland experience an accident with horses at least once a year (Jaeggin et al., 2005). In the USA 65% (Kriss and Kriss, 1997; McCrory and Turner, 2005), 66% in Australia (Abu-Zidan and Rao, 2003), and 75% in England (Chitnavis et al., 1996) riders had an accident related to the fall. In France, according to the report of "Mutualite' Sociale Agricole" (agricultural social insurance), while 2057 cases were evaluated in fields such as horse grooming and stable cleaning, it has been revealed that the proportion of horse-related accidents was %51. These data draw attention to the management of the unexpected reactions of the horses (Newton and Nielsen, 2005) and the choice and timing of the training method (Weeks and Beck, 1996). For this reason, it is important to create positive horse-human interaction, especially from the early age of horses. With the research, agonistic behaviors, which are the basic behavior in experiencing accidents and injuries in human-horse interaction, were examined. At the same time, the durations and types of socialization of horses with other horses and humans were also investigated. Within the scope of the research, the horses were not trained at the stud farm, and when the horses reached at the age of 2, the training started at the Hippodrome. It was thought that not giving training to horses in the early age period and when starting the training in the adult period cut off the intraspecies interactions was compulsive in terms of the adaptation of the horse to the training processes. For this reason, it is thought that starting the training processes with imprinting in the stud farm when the foal is born will have positive results in the training and management practices of the horse. It was seen that humans and horses on the stud farm interact for about 30 minutes a day. In the hippodrome, it was observed that this duration increased to 240 minutes a day. Although there was no paddock system in the hippodrome, it was thought that the increase in the interaction time of the horse with the human gives positive results in terms of the absence of agonistic and/or stereotypic behaviors in the 0-6 months period. Within the scope of the research, it was thought that the increase in the interaction time between the inter-species had positive results on the welfare of the horses. However, considering that horses are herd animals, the importance of intraspecies interactions for equine welfare should be considered. In this research, the training processes of the horses in the hippodrome were carried out by their trainers at the stud farm. It was thought that the establishment of a trusting relationship between horses and humans at an early age had a positive effect on low stereotypic behavior in horses. However, it was thought that the inability of the horses in the hippodrome to interact with intra-species might cause stress in the long term, and this situation could increase the frequency of agonistic and stereotypic behavior.

Budzyinska (2012) investigated the relationship between the level of reactivity to short-term stressful stimuli and behavioral and physiological stress indicators in Arabian mares. As a result of the research, it was revealed that there was a significant relationship between the salivary cortisol level and the behavioral score as a result of the effects of stress. Cortisol concentration in saliva was affected by various environmental factors, such as time of day, time of feeding, or welfare of the horses (Irvine and Alexander, 1994). Bohak et al. (2013), stated that salivary cortisol concentration had a daily circadian rhythm. Considering this, saliva cortisol samples were taken from mares on the same day and at the same time of the day, and they were examined in relation to the behavioral responses of the horses as a physiological stress parameter. Within the scope of the research, the stress levels of horses were examined, there was no significant difference between the horses housed in the stud farm and the hippodrome at rest. It was thought that the fact that the horses housed in the hippodrome were housed for less than 6 months had an effect. It was an important issue in terms of animal welfare to investigate the effect of long-term intra-species social isolation on horses with future studies. It was stated that this situation should be investigated in future studies for horses kept in the hippodrome for more than 6 months. However, in our research, it was determined that the salivary cortisol levels of the horses after the race in the hippodrome increased approximately 7.5 times compared to the pre-race. The increase in plasma cortisol levels in horses may occur as a result of stress and exercise. Boucher and Plusquellec (2019) and Mahmood et al. (2020), suggested that the cortisol increase during an experience is directly related to the acute stress level. In the study of Keidzierski et al., (2014) in which they examined the changes in saliva and plasma cortisol levels during race training, they revealed that lactic acid in the blood, cortisol levels in saliva and plasma increased significantly after the exercise. In the study of Cengiz (2011), it was determined that the pre-exercise cortisol level in horses increased 1.8 times compared to post-exercise. Schmidt et al., (2009) demonstrated a positive correlation between plasma and salivary cortisol concentrations in horses exposed to different stress factors. In a study by Hovey et al. (2021), evaluating the differences in stress-related behaviors and serum cortisol concentrations in horses used in a therapeutic riding program and a university riding program, it was revealed that horses in both groups were exposed to low stressors. They emphasized that serum cortisol is an objective and easy-to-use method for assessing the stress levels of horses. Janczarek et al. (2023) revealed that increased salivary cortisol in horses represents a measure of fear-related stress. Research results reveal that flat races cause stress in horses. In this context, salivary cortisol is a reliable tool for measuring the stress caused by flat races in horses.

Conclusion

Hippodromes are designed for the organizations of races. Because of not include paddocks they are not suitable for horses to live in. As a result of being housed in management systems without paddocks, horses cannot show their social and locomotor behaviors. As a result of the horses' inability to show their natural behavior, one of the 5 freedoms that form the basis of animal welfare is not realized. This may adversely affect horse welfare and health. For this reason, it is a traumatic situation for horses to move from stud farms where the welfare conditions are suitable and where they can show their natural behaviors to hippodromes where they cannot show their intra-species social behaviors. It is recommended that the horses be kept only during the race period, instead of being housed in the hippodrome for a long time, in order to ensure their welfare.

Positive human-animal interaction plays an important role in the training and management system of horses. Horses are herd animals and the presence of people with whom they interact positively helps them feel safe. When horses move to a different management system, the presence of grooms/trainers with whom they have previously established a trusting relationship may prevent the increase in the frequency of agonistic and/or stereotypic behavior. It can also reduce accident and injury rates.

The inability of the horses housed in the hippodrome to exhibit their natural behaviors in intraspecies interactions has the potential to increase the frequency and intensity of agonistic and stereotypical behaviors. Within the scope of the research, it is seen that keeping the box windows of the horses housed in the hippodrome constantly closed, minimizes both intra-species and inter-species interaction. However, considering that horses are both herd animals and owe their lives to their timidity, seeing and hearing other horses and people is important for their welfare.

It is seen that flat-running races have an increasing effect on the cortisol levels of horses. In order for the horses to have a long and healthy sports life, the welfare of the horses should be structured by considering the training, behavior, and management systems with a holistic approach, and intra-species interactions should not be overlooked.

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Conflict of Interest

The author declare no conflicts of interest.

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LIVESTOCK STUDIES

RESEARCH PAPER

Assessment of the Nutraceutical Values of the Leaf of Tapinanthus Globiferus Hosted by the Neem Tree

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Abstract

The objective of this study is to evaluate the nutraceutical values of the leaf of *Tapinanthus globiferus* hosted by the neem tree. The phytochemical composition, antioxidant activities, antidiabetic properties, anti-inflammatory capabilities, and proximate composition of the *Tapinanthus globiferus* leaf powder (TLP) were examined. The results revealed the phytochemical profiles: phenol (501.51 mg/g), alkaloids (408.25 mg/g), saponins (7.16 mg/g), steroids (7.16 mg/g), flavonoids 933.79 mg/g) and tannins (86.53 mg/g); antioxidant assay results: Ferrous chelating activity (51.17%), hydroxyl radical inhibition (55.96%), ABTS (76.09%), DPPH (71.34%) and lipid oxidation inhibition (66.18%); antidiabetic assay results: the α-glucosidase inhibition (75.88%) and α-amylase inhibition (72.14 %); anti-inflammatory assays: antiproteinase activity (72.38%) and albumin denaturation inhibition (44.67%) of TLP. *Tapinanthus globiferus* leaf powder has nitrogen-free extract content of 36.09%, 18.47% crude protein, 13.27% moisture, 12.78% ash, 9.44% crude fibre, and 9.93% crude fat. These findings indicated that TLP had anti-inflammatory, anti-diabetic, and antioxidant properties.

Introduction

Natural foods known as nutraceuticals are renowned for fusing the lines between food and medicine by helping to tackle some of the most serious health issues, including arthritis, cancer, osteoporosis, diabetes, cardiovascular disease, and cholesterol (Das et al., 2012). In addition, a wide variety of products, including processed cereals, processed drinks, isolated nutrients, plant products, diet supplements, and genetically modified items, are included in the category of nutraceuticals (Padmavathi, 2018). The nutraceutical industry's fastest-growing subsectors include dietary supplements and herbal products (Hathcock, 2001, Das et al., 2012). Numerous commonly occurring plants, including eucalyptus, red silk cotton, lemon, henna, spearmint, basil, and mandarin orange, have been suggested to have potential as nutraceuticals (Amat-Ur-Rasool et al., 2020).

The current global ban on the use of antibiotic-supplemented diets in animal production, which was brought about by consumers' growing awareness of the importance of consuming high-quality animal protein, has encouraged the use of medicinal plants or their extracts as a feed supplement in animal production to improve animal performance and play a significant role in potential therapies to improve animal health (Pourhossein et al., 2015; Mahanta et al., 2017). Additionally, nutraceuticals are now acknowledged as a perfect switch to manage a variety of clinical conditions like allergy, blood cholesterol control, inflammation, sleep dysfunction, arthritis, indigestion, depression, hypertension, and malignancies in man as well as human lifestyle disorders like stroke, heart disease, type-2 diabetes, and obesity (Jha et al., 2021).

Tapinanthus globiferus (African mistletoes) is widely distributed (Imarhiagbe, 2021). Birds disperse the seed of this plant, which they then use to parasitize a variety of hosts, including *Azadirachta indica, Citrus sinensis, Cola acuminata, Vitellaria paradoxa,* and *Combretum glotinosum*, among others (Wang et al., 2022). The plant's broad pharmaceutical significance is only now becoming realised. African people refer to the plant as an "all-purpose herb" since the Tapinanthus species, in particular, are used to cure inflammation, hypertension, depression, diabetes, cancer, fever, malaria, and other ailments (Wang et al., 2022). The chemical composition of mistletoe leaf has been extensively studied and reported; however, recent reports indicate that phytochemical or botanical variation exists due to factors such as abiotic conditions, season, diurnal rhythm, ontogeny, diurnal rhythm, and abiotic conditions (Lämke and Unsicker, 2018). As a result, regular or periodic examination and characterization of plant parts are needed to determine their effectiveness in producing results when used as a dietary supplement The majority of therapeutic areas are covered by dietary supplements, including digestion, the prevention of certain cancers, the treatment of colds and coughs, blood pressure, sleep problems, osteoporosis, cholesterol control, diabetes, and depression (Pandey et al. 2010). Therefore, the objective of this study is to evaluate the nutraceutical properties of TLP by exploring the phytochemical profile, antioxidant properties, proximate composition, anti-diabetic, and anti-inflammatory effects of *Tapinanthus globiferus* leaf powder.

Material and Methods

Tapinanthus globiferus leaf powder and Reagents

Fresh Tapinanthus globiferus leaves were collected from the Federal Polytechnic in Ado Ekiti, Nigeria's Teaching and Research Farm of the Agricultural Technology Department. The plant was confirmed by a botanist from the Department of Plant and Biotechnology at Adekunle Ajasin University in Akungba Akoko, Nigeria. After being thoroughly cleansed with fresh water, the samples were drained and allowed to dry in the shade for 14 days. After being ground into *Tapinanthus globiferus* leaf powder (TLP), they were stored at 4°C until analysis. The parameters were examined in three repetitions. Three repetitions of analysis for each parameter were performed on the TLP samples. All of the chemicals of the analytical reagent grade used for chemical analysis were purchased from Sigma-Aldrich.

Quantitative phytochemical analysis of TLP

The methods for determining phenols, alkaloids, saponins, flavonoids, and tannins were highlighted and reported by Oloruntola (2021), whereas Madhu et al. (2016) reported the methods for determining steroids.

Phenols

400 g of TLP received a total of 2000 ml of 70% ethanol, which was then added, shaken for six hours, allowed to stand still for an additional 48 hours, and then filtered through the Whatman No 1 filter paper. A rotary evaporator was employed to vacuum condense the TLP ethanolic extract at 35–40 °C. 200 g of TLP was filtered using Whatman No. 1 filter paper after being immersed in 1000 cc of 70% ethanol and vibrated consistently for 6 hours. The phenolic content of TLP was determined using the Folin-Ciocalteau technique, which Otles and Yalcin (2012) described. 50 mL of TLP extract or standard solution was combined with 250 mL of the Folin-Ciocalteau reaction. Five minutes were spent letting this mixture sit at room temperature in a dim environment. A 750 microliter solution of 7 percent Na_2CO_3 was added at the end of this period. With the use of distilled water, the mixture was diluted to 5 mL. The mixture was next given 120 minutes at room temperature and darkness to react. The absorbance of the standards and samples was calculated at 760 nm. In place of the extract, 50 µl of an 80 percent methanol solution was added to the blank solution. The total phenolic content was calculated using a calibration curve and standards that are equivalent to gallic acid.

Alkaloids

The gravimetric technique was used to determine the alkaloid content of the TLP sample (Adeniyi et al., 2009). The TLP was combined with 50 ml of an acetic acid solution in ethanol (10% w/v). The mixture was vibrated and left alone for around 240 minutes before being sieved. The filtrate was diluted to one-fourth of its initial volume on a hot plate. The alkaloids were then precipitated by applying drops of highly concentrated ammonium hydroxide. The precipitate was rinsed with a 1 percent solution of ammonium hydroxide after being filtered through filter paper. After being dried in an oven for 30 minutes at 60°C, the precipitate was transferred to desiccators and weighed again until it reached a constant weight. The weight of the alkaloids was calculated as a percentage or proportion of the sample weight.

Saponins

The vanillin and concentrated sulfuric acid colourimetric method were used to evaluate saponin content (He et al., 2014). The following ingredients were added to the 0.1 ml of TLP extract: 0.5 ml of 50% ethanol, 4.0 ml of 77% sulfuric acid, and 0.5 ml of freshly prepared vanillin solution. The mixture was allowed to cool to room temperature before being heated in a water bath for 15 minutes to 60 °C. To quantify the absorbance at 545 nm, a UV/Vis spectrophotometer was used. The total amount of saponin in each sample was determined and represented as mg tea saponin equivalent per g (TSE/g DW) using a tea saponin calibration curve.

Steroids

Following reports from Madhu et al. (2016), the level of steroids in TLP was detected. 1 ml of TLP steroid extract was put into 10 ml volumetric flasks. Sulphuric acid (4N, 2 ml) and iron (III) chloride (0.5 percent w/v, 2 ml) were then added after the potassium hexacyanoferrate (III) solution (0.5 percent w/v, 0.5 ml). Before being diluted with distilled water to the necessary concentration, the mixture was heated for 30 minutes at 70-20 °C in a water bath with intermittent shaking. The absorbance was calculated at 780 nm and compared to a reagent blank.

Flavonoids

The Surana et al. (2016) method was used to determine the amount of flavonoids in TLP. A test tube containing 0.50 ml of TLP extract received 0.1 ml of potassium acetate solution, 1.50 ml of methanol, 0.1 ml of aluminium chloride solution, and 2.8 ml of distilled water. The same method, but with distilled water instead of aluminium chloride solution, was used to make sample blanks for extract and rutin standard dilutions (10-100 g/ml). After that, the solutions were filtered using Whatman filter paper (No. 1). Absorbance ratios were measured at 510 nm in contrast to blanks. The total flavonoid concentration was then discovered to be equal to 1 mg of rutin per gramme of the ethanolic TLP extract.

Tannins

The Folin-Ciocalteau method was used to measure the total tannin concentration (Biswas et al., 2020). A volumetric flask was filled with 1 ml of the TLP ethanolic extract, 49 ml of distilled water, 1.7 ml of 75% ethanol, 0.1 ml of metaphosphoric acid, 10 ml of 1.0 mol/ml Na2CO3, and 2.5 ml Folin-Ciocalteu (100 ml). The mixture was thoroughly blended and then let to cool for 15 minutes at room temperature. Then, using a spectrophotometer, the absorbance of the standard solution and TLP extract was determined at 680 nm in comparison to a control. To express the sample's total tannin content as a reference against the standard curve, tannic acid (TA) mg TA/g dry weight was used (R^2 = 0.9972).

Antioxidant activities

Ferrous chelating activity

The techniques employed to determine the ferrous chelating activity of TLP were reported by Ebrahimzadeh et al. (2008). Summarily, 50 l of 2 mM $FeCl_2$ was added to 1 ml of various dosages of the TLP extract (0.2, 0.4, 0.8, 1.6, and 3.2 mg/ml). The reaction was then started by adding 0.2 ml of a 5 mM ferrozine solution. After thoroughly shaking the mixture, it was allowed to stand at room temperature for 10 minutes. At 562 nm, the solution's absorbance was then calculated. The positive control was Na_2EDTA.

$$\% \text{ inhibition} = \frac{(\text{The absorbance of control} - \text{The absorbance of TLP extract})}{(\text{Absorbance of TLP extract})} \text{x}100$$

Hydroxyl radical inhibition

To evaluate the hydroxyl radical inhibition capacity of TLP extract, the guidelines reported by Tijani et al (2012) were adhered to. The reaction mixture contained 1.0 ml of reagent (3.0 mM deoxyribose, 0.1 mM EDTA, 2 mM H_2O_2, 0.1 mM L-Ascorbic acid, and 0.1 mM $FeCl_3.6H_2O$ in 10 mM phosphate buffer, pH 7.4) and various strengths of the extract (50-350 g/ml). 1.0 ml of 1 percent (w/v) TBA (in 0.25 N HCl) and 1.0 ml of 10 percent (w/v) TCA were added after the reaction mixtures had been incubated at 37°C for an hour. The pink chromogen (malondialdehyde-(TBA) adduct) was extracted into 1.0 ml of butan-1-ol before the absorbance was measured at 532 nm against a reagent blank. The reaction mixtures were heated in a bain-marie of boiling water for 20 minutes at 100 °C.

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \text{x}100$$

2,2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS)

A technique described by Turkoglu et al. (2010) and Ozgen et al., (2006) was used to perform the modified ABTS experiment assay. Potassium persulfate was used to make ABTS, which was then dissolved in a buffer solution containing 20 mM sodium acetate (pH 4.5) to achieve an absorbance of 0.700. (0.01 at 734 nm). Then, 100 g/ml concentrations of 1 ml of ABTS + solution and 3 ml of TLP extract in ethanol were mixed. The absorbance was measured 30 minutes after mixing, and at each concentration, the radical scavenging % was calculated in comparison to a blank with no scavenger. The degree of decolourization is assessed using the percentage reduction in absorbance. To draw a standard curve, several ABTS + concentrations were used. The scavenging efficiency of the test chemicals was calculated using the formula below:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance in presence of TLP extract})}{(\text{Absorbance of control})} x100$$

2, 2-diphenyl-1- hydrazine-hydrazyl (DPPH)

The antioxidant activity of the TLP sample was evaluated using the 2, 2-diphenyl-1-picryl-1-hydrazine-hydrazyl (DPPH) radical degradation activity technique (Otles and Yalcin, 2012). The DPPH radical was generated using just pure methanol, and 100 µl of a sample extract or standard solution was combined with 2 µl of a methanolic DPPH solution. This combo was kept in the dark for 20 minutes. The sample absorbance was then measured at 515 nm. A blank solution made entirely of methanol was used. 100 microliters of pure water were substituted for the 100 microliters of extract in the control solution. The antioxidant capacities of sample extracts were evaluated using a calibration curve established with various gallic acid solution concentrations (10-100 ppm).

Lipid peroxidation inhibition

A technique previously reported by Bajpai et al. (2015) was used to determine the TLP extract's ability to reduce lipid peroxidation. The reaction mixture of 1 mM $FeCl_3$, 50 µl of bovine brain phospholipids (5 mg/L), and 1 mM ascorbic acid in 20 mM phosphate buffer was incubated at 37°C for 60 minutes in both the absence and addition of TLP extract (50-250 g/mL) or a control material. Byproducts of the procedure included hydroxyl radicals, which resulted in lipid peroxidation and the production of malondialdehyde (MDA), which was detected by the 2-thiobarbituric acid (TBA) reaction. Calculated was the inhibitory activity proportion.

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{(\text{Absorbance of control})} x100$$

Tapinanthus globiferus leaf proximate analysis

The AOAC method was used to analyse TLP for moisture, crude fat, crude fibre, crude protein, ash, and nitrogen-free extract (AOAC, 2010).

Antidiabetic properties

Alpha-glucosidase inhibitory activity

An assay for assessing the glucosidase inhibitory activity of TLP was described by Dej-adisai and Pitakbut (2015). The glucosidase enzyme converts the substrate, p-nitrophenol-D-glucopyranoside (pNPG), into the yellow product, p-nitrophenol (pNP), which is used to analyse the glucosidase reaction. 50µL of a 10 mM phosphate buffer solution (pH 7) containing 0.2 mg/mL sodium azide and 2 mg/mL bovine serum albumin were added to a well plate.

One unit/mL of Saccharomyces cerevisiae α-glucosidase and 50 L of an 8 mg/mL sample solution were added to the phosphate buffer solution (Type I, lyophilized powder, Sigma, EC 3.2.1.20). The solvent control was a 5 percent DMSO solution, and the positive control was 8 mg/mL of acarbose in each well. The mixes were incubated at 37o C for 2 minutes. 50 microlitres of 4 mM pNPG were then put into the well. The mixture has to incubate for a further five minutes in the same circumstances. For 5 minutes, the pNP was carried out and timed using a microplate reader at 405 nm every 30 seconds. The following linear relationship equation between absorbance and time was used to calculate the velocity (V).

$$\text{Velocity} = \frac{\Delta Absorbance\ at\ 405\ nm}{\Delta\ Time}$$

Each sample's initial reaction's highest velocity was gathered, and the equation below was used to calculate the percentage of inhibition.

$$\% \text{ Inhibition} = \frac{\text{V control} - \text{V sample}}{V\ control} X\ 100$$

Alpha-amylase inhibitory activity

The α-amylase inhibition study was carried out using the 3,5-dinitrosalicylic acid (DNSA) method (Wickramaratne et al., 2016). To create concentrations ranging from 10 to 1000 g/mL, the TLP extract was treated with at least 10 percent dimethylsulfoxide and then diluted in buffer ((NaCl (0.006 M, Na_2HPO_4/NaH_2PO_4 (0.02 M, at pH 6.9). 200 µL of extract and 200 µL of amylase solution were mixed and incubated at 30 °C for 10 minutes. After that, each tube received 200 µL of the starch solution (1 percent in water (w/v)) and was incubated for 3 minutes. The reaction was stopped by adding 200 µL DNSA reagent (12 g sodium potassium tartrate tetrahydrate in 8.0 mL 2 M NaOH and 20 mL 96 mM 3,5-dinitrosalicylic acid solution) to a water bath at 85–90 °C and boiling for 10 minutes. The mix was cooled to room temperature and diluted with 5 mL distilled water before being analysed with a UV-Visible spectrophotometer at 540 nm. By substituting 200 µL of buffer for the plant extract, a blank with 100% enzyme activity was created. In the absence of the enzyme solution, a blank reaction was generated using the plant extract at each concentration. As a positive control sample, acarbose (100–200 µg/mL) was employed, and the reaction was conducted in the same manner as the plant extract reaction. Using the equation below, the inhibitory activity of -amylase was calculated and reported as a percentage of inhibition. By plotting the percentage of α-amylase inhibition versus the extract concentration, the IC50 values were determined.

$$\% \ \alpha - \text{amylase inhibition} = 100 \ \text{X} \frac{\text{Absorbance } 100\% \text{ Control} - \text{Absorbance Sample}}{\text{Absorbance } 100\% \text{ Control}}$$

Anti-inflammatory activities

Albumin denaturation inhibition

The assay was carried out as outlined by Osman et al., (2016). Ibuprofen and diclofenac, two positive standards, were produced at a concentration of 0.1 percent each (1.0 mg/ml), along with the TLP extracts. Each mixture's reaction vessel was made up of 1000 µl of the test extract, 1400 µl of phosphate-buffered saline, and 200 µl of egg albumin. As a negative control, distilled water was utilised in place of the extracts. The mixtures were then heated for 5 minutes at 70°C after 15 minutes of incubation at 37°C. Their absorbances at 660 nm were measured after cooling. This formula was used to determine the protein denaturation inhibition percentage:

$$\% \text{ Denaturation inhibition} = \left(1 - \frac{\text{Absorbance reading of the test sample}}{\text{Absorbance reading without test sample } (-\text{ve control})}\right) * 100\%$$

Antiproteinase activity

The test was performed as outlined by Rajesh et al., (2019). 1 ml of 20 mM Tris-HCl buffer (pH 7.4), 0.06 mg of trypsin, and 1 ml of the test sample with varying concentrations (100–500 g/ml) were all included in the reaction mixture (2 ml). For five minutes, the mixture was kept heated at 37°C. 1 ml of 0.8 percent (w/v) casein was then added to the mixture. A further 20 minutes were spent keeping the mixture heated. To stop the process, 2 ml of 70% perchloric acid was added to the mixture. The murky suspension was then centrifuged after that. The supernatant's absorbance was then measured at 210 nm using a buffer as a blank. Three times the experiment was conducted. The following formula was used to calculate the % inhibition of proteinase inhibitory activity:

% inhibition = (Abs control-Abs sample)*100/Abs control

The TLP proximate composition analysis

The TLP was analyzed for moisture, crude fat, crude protein, ash, and nitrogen-free extract using the AOAC method (AOAC, 2010).

Statistical analysis

The average mean was the statistical method used in this study. Each assay was performed three times, and the results' average mean was reported. To better comprehend the average mean, bar graphs were made in Excel.

Results

The secondary metabolites, or phytochemicals, that plants synthesize as a defence against reactive oxygen species play a part in determining the phytogens' nutraceutical qualities. The phytoconstituents analysis as shown in Figure 1 reveals phytochemical profiles of TLP: phenol (501.51

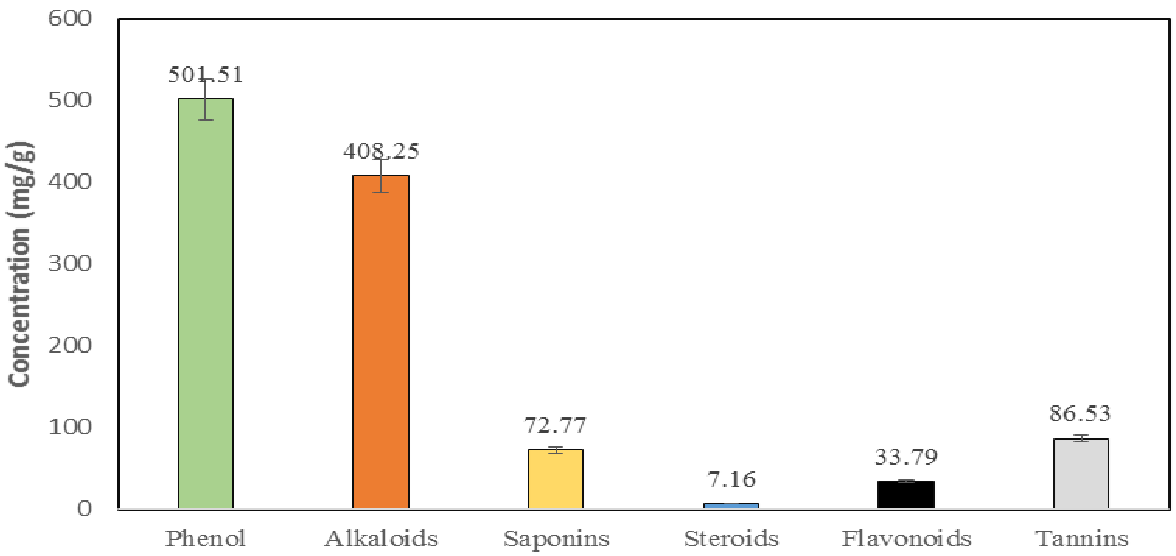
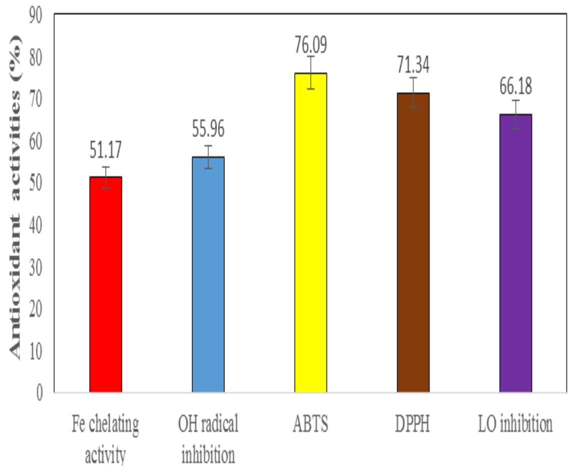
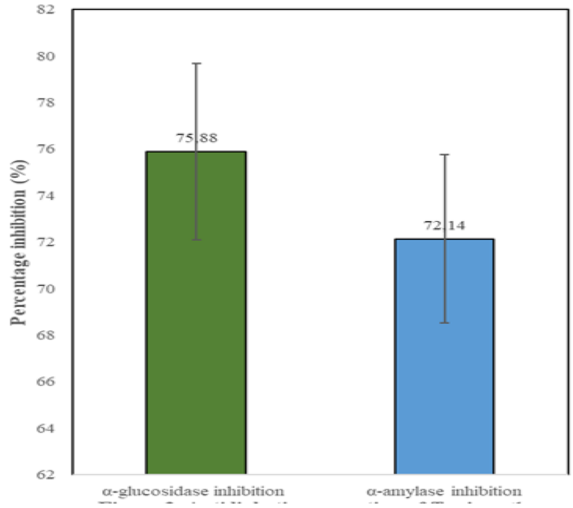


Figure 1. Phytoconstituents of *Tapinanthus globiferus* leaf powder



Figüre 2. Antioxidant assays of *Tapinanthus globiferus* leaf powder
Fe: Ferrous; OH: Hydroxyl; ABTS: 2,2-Azino-Bis-Ethylbenzothiazoline-6-Sulfonic Acid; DPPH: 2,2-dicrylhydrazyl; LO:



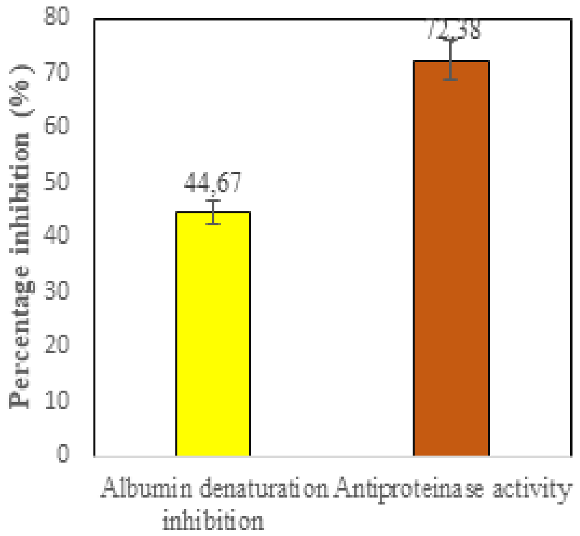
Figüre 3. Antidiabetic properties of *Tapinanthus globiferus* leaf powder

mg/g), alkaloids (408.25 mg/g), saponins (7.16 mg/g), steroids (7.16 mg/g), flavonoids 933.79 mg/g) and tannins (86.53 mg/g). This shows that TLP might have a place in nutraceuticals. TLP's content of phenol and flavonoids, in particular, may influence cellular functions like apoptosis, gene expression, and intracellular signalling that may have anti-atherogenic and anti-carcinogenic effects (Duthie et al., 2003). Additionally, the substantial amounts of saponins and tannins in TLP also demonstrate the phytogens' nutraceutical properties because the aforementioned phytochemicals have been identified as significant bioactive plant components with anticancer, anti-bacterial, antioxidant, and antimicrobial properties (Yildirim and Kutlu, 2015). The results of this study's phytochemical examination of TLP hosted by neem trees are different from those for total phenol (155.01 mg/g), flavonoids (15.22 mg/g), alkaloids (6.75 mg/g), saponins (220.83 mg/g) and tannins (10.10 mg/g) recorded for the leaf of mistletoe hosted by kola nut trees being reported by Oloruntola and Ayodele (2022). These variances may result from various plant sources, including host plant species and various processing techniques (Ishiwu et al., 2003; Oloruntola, 2022).

It is established that consuming phytogens with high antioxidant phytochemical concentrations raises the serum's antioxidant capacity (Zhang et al., 2015). The outcomes of the TLP antioxidant assays in this study: Ferrous chelating activity (51.17%), hydroxyl radical inhibition (55.96%), ABTS (76.09%), DPPH (71.34%) and lipid oxidation inhibition (66.18%) (Figure 2) further unveils the antioxidant properties of TLP. The H_2O_2 scavenging activity, iron-reducing capacity, iron binding ability, 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity, and OH scavenging activity are the most widely used antioxidant tests for determining the antioxidant activity of food or feed (Adjimani and Asare, 2015). The phytogen is a potential candidate for an antioxidant dietary supplement based on its ferrous chelating activity, hydroxyl radical inhibition, ABTS, DPPH, and lipid oxidation inhibition properties. When compared to *Justicial carnea* leaf powder, TLP's levels in several antioxidant assays are higher: hydroxyl radical inhibition (55.96% vs. 42.81%), ABTS (76.09% vs. 28.49%), DPPH (71.34% vs. 54.35%), and lipid oxidation inhibition (66.18% vs. 62.21%) (Oloruntola et al., 2022). The antioxidant activities of TLP are beneficial because consuming antioxidants is thought to lessen the harm that oxidative stress causes, such as cancer, ageing, atherosclerosis, and vision loss (Salminen et al., 2014).

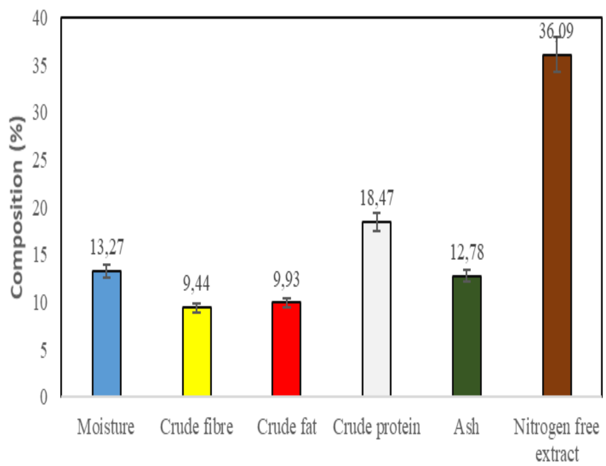
The α-glucosidase inhibition (75.88%) and α-amylase inhibition (72.14 %) activities of TLP (Figure 3) in this study suggest the phytogens could be a potential antidiabetic feed or food supplement. These antidiabetic properties could be due to the phytoconstituents of TLP, particularly the polyphenols (e.g. phenol and alkaloids) concentrations (Sekhon-Loodu and Rupasinghe, 2019) of TLP. In addition to their antioxidant benefits, dietary polyphenols have been shown to exhibit anti-hyperglycemic properties through their binding to glucose transporters and competitive suppression of digestion enzymes (Lacroix and Li-Chan, 2014). Dietary starch is broken down by the hydrolyzing enzymes α-amylase and α-glucosidase into glucose, causing a postprandial glucose spike. Therefore, one of the main methods to treat hyperglycemic situations is to limit the activities of α-amylase and α-glucosidase (Sekhon-Loodu and Rupasinghe, 2019). It is known that the polyphenols interact with the enzyme by non-specific binding, which inhibits enzyme activity (Wang et al., 2013). The inhibition of α-glucosidase and α-amylase recorded for TLP in this study is greater than the corresponding inhibitions of 65.82% and 65.96% recorded for Jacobinia leaf powder (Oloruntola et al., 2022).

Figure 4 depicts the anti-inflammatory properties of *Tapinanthus globiferus* leaf powder. The



Figüre 4. Anti-inflammatory properties of *Tapinanthus globiferus* leaf powder

phytochemical constituents aid in the expression of botanicals' anti-inflammatory properties (Leyva-Jimenez et al., 2020). According to the percentages of antiproteinase activity (72.38%) and albumin denaturation inhibition (44.67%) that were measured for TLP in this study, the phytogens may have anti-inflammatory effects when used as dietary supplements. This is of health benefit because, with an anti-inflammatory diet, the risk of heart disease, inflammatory bowel illnesses, arthritis, Alzheimer's, psoriasis, and other conditions could be decreased (Stromsnes et al., 2021). The antiproteinase activity and albumin denaturation inhibition properties of TLP could be due to its phenolic content because correlations were reported to exist between the anti-inflammatory activities of phytogens and their phenolic contents (Naz et al., 2017). Phenolic chemicals can reduce inflammation by either inhibiting the generation of pro-inflammatory mediators or their activity (Ambriz-Perez et al., 2016). The percentage of antiproteinase activity and albumin



Figüre 5. Prominate composition of *Tapinanthus globiferus* leaf powder

denaturation inhibition recorded for TLP are lower than 79.17% and 62.71%, respectively in the *Juglans regia* kernel (Oloruntola, 2022).

The proximate composition influences the suitability of food and supplements for dietary inclusion (Oloruntola et al., 2022). TLP has a nitrogen-free extract content of 36.09%, 18.47% crude protein, 13.27% moisture, 12.78% ash, 9.44% crude fibre, and 9.93% crude fat, according to Figure 5. The proximate profile of TLP indicates that it may serve as a source of protein and dietary minerals when used as a feed ingredient, dietary supplement, or additive in man and animal nutrition.

Conclusions

TLP may therefore have anti-inflammatory, anti-diabetic, and antioxidant effects. Therefore, TLP is advised for use in feeding studies using an animal model as a dietary phytogenic and nutraceutical supplement.

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LIVESTOCK STUDIES

REVIEW ARTICLE

Molecular Mechanisms Affecting Development of Animal Fibers and Some Studies on Goats

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Abstract

Natural fibers are of two types as vegetable and animal origin. Animal fibers have contributed to the development of the economic structures of the countries and the continuation of their traditions since ancient times and continue to do so. Various studies have shown that the properties of the fiber produced by the hair follicle are of great importance in determining fiber quality and quantity. Hair follicles have been developed in an environment where various molecular signals are effective. In this context, the investigation of molecular mechanisms affecting hair follicle development has come into prominence and it is seen that it has become the focus of studies. In this review, it has been tried to explain the importance of animal fibers and the molecular mechanisms affecting hair follicle morphogenesis and cycle by providing some examples from the studies on two goat breeds, Angora goat and cashmere goat, which are valuable in terms of animal fiber, leading to provide a preliminary information to the studies which has become the focus of today.

Introduction

Natural fibers are two types: plant and animal. Plant fibers produced at industrial level are cotton, kapok, flax, hemp, jute, sisal, manila hemp, coco fiber, while animal fibers are fleece, silk, mohair, cashmere, Angora rabbit wool, goat top coarse fibers and fibers obtained from Asian and South American camels (Dellal *et al*., 2010).

As environmental pollution has reached serious levels, the need, demand and interest in biodegradable, environmentally friendly products and sustainable production has increased. This situation is also reflected in the interest in natural fibers and therefore animal fibers from environmentally sensitive products in the textile sector (Selli *et al*., 2018). Animal fibers constitute the raw material of both traditional and industrial fiber processing systems (Gul *et al*., 2023). With this feature, it has contributed to the development of the economies and traditions of countries since time immemorial and continues to do so (Dellal, 2021).

Animal fibers are generated by hair follicles in the skin. They have specific growth, development and self-renewal mechanisms (Zhang *et al*., 2009). Hair follicles are considered as mini-organ formed as a result of neuroectodermal-mesodermal interaction (Schmidt-Ullrich *et al*., 2005).

The hair follicle is first formed during the embryonic period, and its growth is completed in this period as well (Dellal, 2021). This morphogenesis that occurs during the embryonic period includes three stages: induction, organogenesis and cytodifferentiation (Houschyar *et al*., 2020).

After birth, hair follicles are developed in a cycle known as hair follicle cycle. This cycle includes anagen (growth), catagen (regression) and telogen (relative stagnation) phases. Each phase of hair follicle cycle has its own unique mechanism of gene activation and silencing. The transition from one phase to another is controlled by transcription factors and enzymes recognized by the local signaling environment, cytokines, hormones, neurotransmitters and key mediator molecules (Yuan *et al*., 2013). Hair follicle development and hair growth are mediated by ectodermal-mesodermal interactions before and after birth, respectively (Sennett and Rendl, 2012).

To the best of our knowledge, most of signaling molecules that regulate hair follicle morphogenesis belong to Wnt pathway (Li *et al*., 2004), fibroblast growth factor (FGF) family, tumor necrosis factor (TNF) family (Millar, 2002), bone morphogenetic protein (BMP) family (Thomadakis *et al*., 1999), Sonic hedgehog (Shh) transduction pathway (McMahon *et al*., 2003), transforming growth factor (TGF) family (Ullrich and Paus, 2005) and Notch transduction pathway (Crowe *et al*., 1998). Some of them show activator and some inhibitory effects (Shang *et al*., 2021). However, the exact nature, timing, and intersections of these signaling pathways remain unclear (Sennett and Rendl, 2012).

miRNAs and lncRNAs are other molecules that regulate hair follicle development and regeneration in mammals. Liu et al. (2012) identified 316 conserved miRNAs and 22 novel miRNAs in Mongolian cashmere goats and predicted that miR-203 may play an important role in the development of skin and hair follicles. miR-203 was confirmed to regulate the development of cashmere goat hair follicle by targeting NAE1 and DDOST gene (Ma *et al*., 2021).

Expression analysis showed that lncRNA-000133 was more highly expressed in the anagen phases than in the telogen phase in cashmere goats. This suggests that this long non-coding RNA (lncRNA-000133) may be involved in formation and growth of cashmere fiber and restructuring of secondary hair follicles. Overexpression of lncRNA-000133 led to relatively significant increase in the expression of ALP, ET-1, LEF1 and SCF genes in dermal papilla cells, suggesting that lncRNA-000133 contributes to inductive property of dermal papilla cells (Zheng *et al*., 2020).

Most studies indicate that hair follicle characteristics and/or genetic regulatory mechanisms directly affect fiber production and quality (Nixon *et al*., 1991; Millar, 2002; Fu *et al*., 2020; Han et al., 2018; Su *et al.,* 2018; Wang *et al*., 2021; Wang *et al*., 2017; Qiao *et al*., 2016; Shang *et al*., 2021; Wang *et al*., 2015; Wu *et al*., 2022; Pazzaglia *et al*., 2019; etc.). More knowledge and better understanding of development and biological properties of the fiber formed by the hair follicle can provide important approaches to obtain the fiber with the desired properties (Gao *et al*., 2016; Arzik *et al*., 2023a). Fiber fineness and quantity are the most important factors determining its economic value (Arzik *et al*, 2023b). Therefore, knowing the regulatory effect of genes, signaling pathways and all mechanisms affecting hair follicle development on animal fiber is great importance in obtaining finer fiber.

Angora goat, which is an important genetic resource of our country, and cashmere goat are two goat breeds that are mainly bred for their fiber. Although there are mostly studies on cashmere goat, studies on these two goat breeds to determine the molecular mechanisms affecting hair follicle development are very valuable and it is seen that more studies are needed to obtain finer fiber.

In this review, the molecular mechanisms that provide hair follicle development and examples of studies on cashmere and Angora goats are given and it is aimed to provide preliminary information for future studies on this subject.

Molecular Mechanism of Hair Follicle Morphogenesis

Hair follicle morphogenesis consists of induction, organogenesis and cytodifferentiation and is mediated by signaling molecules.

Induction: The first structure formed as a result of thickening of the epidermis is called placode. The first dermal signal that enables the formation of this structure comes from the mesenchyme (Rishikaysh *et al*., 2014). In the absence of β-catenin, it has been determined that the placode structure does not form (Huelsken *et al*., 2001). However, the nature of the signal that initiates placode formation has not been fully elucidated (Wang *et al*., 2012). Wntless (Wls) protein is involved in Wnt secretion (Huang *et al*., 2012). Wnt signaling is another signal that is effective in placode formation.

Another important signal during the induction phase is Eda/Edar/Nuclear Factor Kappa-B (NF-κB) (Durmowicz *et al*., 2002; Laurikkala *et al*., 2002). Follistatin (FS), fibroblast growth factors (FGFs), MSX1 and MSX2, Noggin and TGF-β2 have a positive effect while BMPs have negative effect on placode formation (Wang *et al*., 2012). In addition, keratinocyte growth factor (KGF) and epidermal growth factor (EGF) block hair follicle formation (Richardson *et al*., 2009). Noggin signaling is important for the maintenance of hair follicle formation. Noggin knock-out mice were found to have a significant delay in hair follicle induction. Noggin eliminates the inhibitory effect of BMP-4 (Botchkarev *et al*., 1999).

Organogenesis: After the formation of the placode structure, some of the cells in the mesenchyme cluster to form the ‘dermal condensate’ under the influence of the epithelial signal from the placodes. Wnt signaling acts as an epidermal signal in the formation of the dermal condensate (Millar, 2002). As a result of the so-called ‘second dermal signal’ from the dermal condensate, placode proliferate, invade the dermis and surround the dermal condensate, forming a structure called the dermal papilla (Hardy, 1992). The main signal involved in dermal papilla formation and maturation is the Shh signal (Chiang *et al*., 1999; St-Jacques *et al*., 1998). The proliferation of placodes and the formation of dermal condensate structure indicate that the organogenesis phase has started. EDA (Ectodysplasin-A)/ EDAR/ NF-ƘB is also important signaling pathway in the progression of organogenesis. EDA/ EDAR/ NF-ƘB signaling is involved in the activation of epithelial Shh (Sonic hedgehog) and cyclin D1 expression (Schmidth-Ullrich et al., 2006). The EDA/EDAR signaling pathway can be summarized as EDA→ EDAR→ EDARADD→ NF-κB→ Shh→ Cyclin D1. The expression of Cyclin D1 is initiated by Wnt10b or Shh signaling (Andl *et al*., 2002; Schmidth-Ullrich and Paus, 2005; Laurikkala *et al*., 2002). TGF-β2 signaling provides the basis for transient induction of Snail, a transcription factor, and activation of the Ras-mitogen-activated protein kinase (MAPK) pathway in the hair bud (Jamora *et al*., 2005).

Cytodifferentiation: With cytodifferentiation, the entire hair follicle is formed. It has been determined that many signaling pathways are effective in the differentiation phase. Wnt/β-catenin and Lef1 signaling are among the signals that are important in hair structure differentiation (Botchkarev and Paus, 2003). Notch signaling pathway also plays a role specific differentiation of the hair structure. It has been determined that overexpression of Notch-1 causes the hair medulla to be abnormal and the fiber cover to be wavy and shiny. Notch and Jagged-1/2, the ligand of Notch-1, are effective in the differentiation of the inner root sheath and hair shaft (Lin *et al*., 2000; Kopan and Weintraub, 1993; Powell *et al*., 1998; Favier *et al*., 2000). Wnt5a mediates Notch signaling by facilitating the expression of the FoxN1 gene. While Wnt signaling has a positive effect on the Notch signaling pathway, recent data suggest that *Notch* has an antagonist effect on Wnt (Hayward et al., 2005; Proweller *et al*., 2006). FoxN1 regulates both the differentiation of hair follicle keratinocytes (Mecklenburg *et al*., 2001) and signals pigment transport from melanocytes to keratinocytes in the hair cortex (Weiner *et al*., 2007). Notch can contribute to hair follicle development in three ways: lateral inhibition, the formation of boundaries of the structures that make up the hair follicle, and the decision of which cell line to become. Notch mediates differentiation by suppressing p63 expression (Nguyen *et al*., 2006). Notch, Wnt and vitamin A are part of interconnected pathways involved in the selection of epidermal cell line (Watt *et al*., 2006).

Molecular Mechanisms Affecting the Hair Follicle Cycle

Our knowledge of the molecular mechanisms involved in the hair follicle cycle is mostly based on studies with mice. The signals that initiate the anagen phase are Wnt/β-catenin, BMP antagonists and sonic hedgehog (Cotsarelis and Botchkarev, 2008; Stenn and Paus, 2001; Paus and Foitzik, 2004; Hebert *et al*., 1994). The effect of hepatic growth factor (HGF), insulin-like growth factor-1(IGF-1) and vascular endotelial growth factor (VEGF) are reported to be important in maintaining the anagen phase (Paus and Foitzik, 2004).

The transition from anagen to catagen Keratin 17 and TNF-α signaling and is controlled by vitamin D receptor (VDR), retinoic acid receptor and Hairless (Hr) transcriptional receptor (Chuma *et al*., 2012; Wu *et al*., 2014). Neurotrophins such as NT3, NF4 and BDNF, interleukin-1β, TGF-β1 are reported to induce catagen (Foitzik *et al*., 2005). FGF-5 is thought to be critical in this transition (Oh *et al*., 2016). Because it has been determined that in case of any defect in FGF-5, the transition from anagen to catagen is delayed. This results in the formation of an excessively long hair structure called the ‘angora’ phenotype (Rishikaysh *et al*., 2014; Hébert *et al*., 1994). Hairless, regulates transcription by interacting with VDR. In the absence of these regulators, dermal cysts are formed (Teichert *et al*., 2010).

Although the telogen is characterized as the resting phase, it is the phase in which some major changes occur (Lin *et al*., 2004). Telogen consists of two sub-phases: refractor phase and competent phase. In the refractor phase, BMP2/4 is upregulated, and hair follicles show resistance to growth stimuli. In the competent phase, there is susceptible to signals that will initiate anagen and efficiency of BMP signaling decreases (Plikus *et al*., 2008). It has also been found that the expression of estrogen receptor is significantly upregulated during telogen (Ohnemus *et al*., 2005).

Some Studies with Goats

Diao *et al*. (2023) suggested that secondary hair follicle development in kids determines cashmere quality in adults and conducted an experimental study to support this idea. They suggested that secondary hair follicle development is completed at five-six months of age and FGF2, FGF21 and BMP7 may play a regulatory role in this development. They stated that hair follicle characteristics can be used as a parameter for breeding selection of cashmere goats at the age of six months of age.

In the study by Cinar-Kul *et al*. (2022), skin biopsy materials were taken from Angora goats in anagen and telogen phases. Expression levels of BMP-2, FGF-5, HOXC13, KAP9.2 and TGFBR2 genes were analyzed. It was determined that TGFBR2, FGF-5 and BMP-2 genes were significantly upregulated in the anagen phase, while the expression of KAP9.2 gene did not differ between the two phases. HOXC13 gene showed a high level of expression in the anagen phase, while expression of this gene was not detected in the telogen phase. The researchers stated that HOXC13 may be one of the factors underlying the shinier and silky and non-medular hair structure of mohair.

Wu *et al*. (2022) took skin samples from Jiangnan cashmere goats at three phases of the hair follicle cycle. Genes affecting hair follicle cycle and development and the interactions of mRNAs and lncRNAs associated with these genes were investigated. They identified 228 differentially expressed mRNAs and 256 lncRNAs. Several key genes including CHST1, CDKN1A, DEFB103A, SH3BP4, GAREM1, GSK-3β, KRTAP9-2, KRT2, KRT39, YAP1, S100A7A, FA2H, LOC102190037, LOC102179090, LOC102173866, FAM167A, FAT4 and EGFL6 have been shown to have important roles in hair follicle development and cycling. These genes are associated with Wnt/β-catenin, ERK/MAPK, NFκB/p38MAPK, TGFβ, Sonic hedgehog, mTORC1, caspase-1 and interleukin (IL)-1a signaling pathways.

In the study by Liu *et al*. (2022), melatonin was implanted into cashmere goats, and then skin samples were taken, and transcriptome sequencing and weighted gene co-expression analysis (WGCNA) were performed. One of the 14 co-expression modules identified by WGCNA was found to be associated with cashmere development after melatonin implantation. KEGG analysis revealed that the initial onset of melatonin-induced cashmere development was mainly related to the signaling pathway regulating stem cell proliferation and the Hippo, TGF-β and MAPK signaling pathways. Analysis of differentially expressed genes identified six hub genes including BMPR2, BMPR1A, PDGFRA, PPP2R1A, SMAD1 and WNT5A.

Zhao *et al*. (2022) investigated the underlying difference between fine ad coarse fiber in Tibetan cashmere goats by using proteomic and transcriptomic data. A proteomics approach identified 1.980 proteins. After comparative analysis of cashmere with coarse fiber and extremely fine fiber, 29 differentially expressed proteins (DEPs) were identified, such as APOH, AEBP1, CP, CPB2, CTSZ, GANAB, GLB1, GPR142, VTN, IMPA1, and HMCN1. Functional enrichment analysis of these DEPs showed that they are involved in cell redox homeostasis, oxidation-reduction process, metabolic, Wnt, PI3K-Akt, MAPK and signaling pathways. Transcription factor enrichment analysis revealed that the proteins mainly belonged to NF-κB family, HMG (high mobility group) family and CSD family. Western blotting confirmed the protein abundance of four DEPs (AEBP1, GC, GPR142 and VTN) and suggested that these are the most potential candidate genes for cashmere traits in Tibetan cashmere goats.

Işık *et al*. (2021) analyzed the genetic variation KAP1.1, KAP1.3 and K33 genes by DNA sequencing method by taking samples from Angora, Norduz, Honamlı, Kilis and Hair goat breeds of our country. A total of 59 nucleotide variations and indels (insertions/deletions) in KAP1.1 gene, 15 nucleotide variations and indels in KAP1.3 gene, 16 nucleotide variations and indels in K33 gene were determined in the analyzed samples. Keratin-mediated filament proteins (KIF or KRT) and keratin-associated (KAP) proteins play an important role in determining the structural and mechanical properties of hair and mohair fibers. Considering this situation, it was stated that there is a need to determine the relationship between the variation detected in KAP1.1, KAP1.3 and KRT1.2 genes and mohair yield and quality in this study.

In a study by Li *et al*. (2021), the expression profiles of long non-coding RNAs (IncRNAs) were analyzed using Zhongwei goats aged 45 and 108 embryonic days. In total, 46.013 mRNAs and 13.549 lncRNAs were identified, of which 352 mRNAs and 60 lncRNAs were differentially expressed. After functional enrichment analysis, it was determined that the genes targeted by lncRNAs were mainly involved in PI3K-Akt, arachidonic acid metabolism, cAMP, Wnt and MAPK signaling pathways. When qRT-PCR and WGCNA results were evaluated together, it was stated that LOC102172172600 and LOC102191729 may affect hair follicle development and wool curvature. KEGG pathway analysis showed significant enrichment of pathways including MAPK, TNF and PI3K-Akt, which play important roles in the regulation of hair follicle development.

Wang *et al*. (2021) attempted to determine the mechanism of periodic development of the hair follicle. With WGCNA, 10 co-expression modules were obtained, including 7.689 protein-coding genes. Of these, six were considered to be significantly associated with hair follicle development. In addition, functional enrichment analysis was performed for each module, and it was shown that it is closely related to ECM-receptor interaction, focal adhesion, estrogen signaling pathway, PI3K-Akt signaling pathway, etc. When this information was combined with the analysis of differentially expressed genes, 12 hub genes (AQP3, ANTKMT, COL1A1, C1QTNF6, COL1A2, DERL2, FA2H, KRTAP3-1, KRTAP11-1, NDUFS5, MRPL14, and XAB2) were selected as candidate markers. It has been stated by the relevant researchers that these genes can be used in the improvement of cashmere fiber production.

In the study by Ahlawat *et al*. (2020), transcriptome pattern of skin samples from Changthangi goats with fiber-type and Barbari goats with meat-type was examined to unravel gene networks and metabolic pathways that may contribute to cashmere development. Functional annotation and enrichment analysis identified significantly enriched pathways such as keratinization, formation of cornified envelope and developmental biology. It was observed that the expression of genes related to keratins (KRTs) and keratin-related proteins (KRTAPs) was much higher in Changthangi goats. A number of transcription regulatory genes involved in hair follicle keratin synthesis, such as ELF5, GPRC5D, PADI3, HOXC13, FOXN1 and LEF1 were upregulated in cashmere producing goats. It was suggested that positive regulation of Wnt signaling pathway and negative regulation of Oncostatin M signaling pathway may contribute to hair follicle development and hair shaft differentiation in Changthangi goats.

Fu *et al*. (2020) investigated the role of lncRNAs and mRNAs in regulating cashmere fineness of Tibetan Cashmere goats. RNA sequencing was performed by taking skin samples from goats with fine-type and coarse-type fiber in the anagen phase. 2.059 candidate lncRNAs (1.589 annotated lncRNAs, 470 novel lncRNAs) and 80 differentially expressed (DE) lncRNAs were identified and their potential targets were predicted. Besides, 384 of 29.119 mRNAs were identified as differentially expressed. Several key genes such as KRT26, KRT28, KRT39, IFT88, JAK3, NOTCH2 and NOTCH3 and a number of lncRNAs including ENSCHIT00000009853, MSTRG.16794.17, MSTRG.17532.2 were shown to be potentially important in regulating cashmere fineness.

Hui *et al*. (2020) investigated the potential effect of miRNAs on cashmere fineness. KEGG enrichment analysis showed that miRNAs significantly enriched progesterone-mediated oocyte maturation, endocytosis, PI3K-Akt and Wnt signaling pathways from other pathways. A total of 27 miRNAs were randomly selected from different genotypes and validated by qPCR. Among these miRNAs, miR-101 was found to negatively regulate cashmere fineness at the lowest rate, while miR-30a-5p and miR-30e-5p negatively regulated it at the highest rate.

Jin *et al*. (2020) genotyped Inner Mongolian cashmere goat and Liaoning cashmere goat which are known for their cashmere yield and Huanghuai goat using Illumina Caprine 50K SNP array and 53.347 single nucleotide polymorphisms (SNPs) were obtained. 222 candidate genes were identified in Inner Mongolia goats and 173 in Liaoning cashmere goats. Several genes such as CSN3, FOXP1, LRRC14, TRPS1, WDR74, SPTLC3, IGF1R, PADI2, WNT10A and were found to be associated with hair follicle development. 20 genes were identified in both cashmere goat breeds. Among the identical genes, WNT10A and CSN3, which are associated with hair follicle development, were potentially involved in cashmere production. Cashmere-related genes were selected and KEGG analysis showed that these genes were clustered in follicle-related pathways. Further analysis showed that these genes were found in pathways regulating stem cell pluripotency, basal cell carcinoma, TGF-β signaling, melanogenesis, Wnt signaling, TNF signaling and PI3K-Akt signaling. The researchers stated that these findings could improve molecular breeding of cashmere goats in the future.

In the study by Su *et al*. (2020), biopsy materials were taken and analyzed in three phases of the secondary hair follicle cycle in Inner Mongolian cashmere goats. There were 29 down-regulated and 22 up-regulated differentially expressed genes (DEGs) in the anagen-catagen phase group, 117 down-regulated and 326 up-regulated DEGs in the catagen-telogen phase group, 582 down-regulated and 197 up-regulated DEGs in the telogen-anagen phase group. As a result of GO and KEGG analyses with differentially expressed genes, FGF5, FGFR1 and RRAS were found to have an impact on the hair follicle growth cycle. The interactive network control chart showed that FGF5, FGFR1 and RRAS are localized to important sites in the MAPK signaling pathway.

Han *et al*. (2018) aimed to determine the differential expression levels of FoxN1, FoxE1 and FoxI3 genes at different periods of fetal development (45, 55 and 65 embryonic ages) and adulthood in Mongolian cashmere goats and to provide new information on genes related to hair follicle development. It has been reported that FoxN1, FoxE1 and FoxI3 genes in the Fox gene family may play a role in hair follicle formation, follicle growth and development.

Zhou *et al*. (2018) tried to determine lncRNA, miRNA and mRNAs that are effective in anagen and catagen phase. lncRNA and miRNA libraries were created by taking biopsy samples from cashmere goats from anagen (September) and catagen (February). 1,122 known and 403 novel lncRNAs were identified, 173 of which were found to be differentially expressed between anagen and catagen. In addition, 3.500 gene-encoding transcripts that are differentially expressed between these two phases were identified. 411 known miRNAs and 307 new miRNAs were identified. Of these, 72 were found to be differentially expressed. It was also known that catagen-inducing factors such as TGFβ1 and BDNF are regulated by miR 873 and lnc108635596 in lncRNA-miRNA-mRNA networks.

Peng *et al*. (2017) carried out the transcriptome analysis of genes expressed in the skin of goats with three different undercoat colors: white, brown, and gray. It was found that the MC1R gene was not differentially expressed in all three coat colors. It was stated that DCT, PMEL, TRPM1 and TYRP1 genes may have an important role in the formation of brown undercoat color, and ELOVL3 and PMEL genes may play an important role in the formation of gray undercoat color. It was stated that the fact that ASIP gene only in goats with white undercoat color seems to be important in the maintenance of this undercoat color, and these results can be used to increase our knowledge in understanding the molecular background involved in the pigmentation of undercoat color.

Conclusion

Angora goat and cashmere goat are two goat breeds that are mainly raised for their fiber. At the same time, Angora goat is in an important genetic resource of our country and cultural heritage. When the existing studies are evaluated, it is seen that there are limited studies to elucidate the molecular mechanisms affecting the quality and quantity of mohair fiber, which are mostly concentrated on increasing cashmere yield and quality, and there is great need for studies in this field. In addition, the studies needed to obtain fine mohair fiber will contribute to the textile industry and rural development in terms of our country. By making animal fiber production sustainable, it will also contribute to the conservation of Angora goat, which is an important genetic resource.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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LIVESTOCK STUDIES

RESEARCH PAPER

Determination of the Current Status of Buffalo Breeding in the Çaycuma District of Zonguldak

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Abstract

This study was carried out to determine the breeding practices, important income sources and the future of water buffalo farming in Çaycuma district of Zonguldak province. For this purposes, 36 breeders were determined from the villages of Çaycuma district to constitute the buffalo population in the region. It is predicted that these breeders in villages in different parts of the district will reveal the current situation of water buffalo breeding in Çaycuma district and have sufficient material for this. First of all, a survey study was conducted with the business owners through face-to-face interviews. In addition, the data of statistical institutions were used. The data were analyzed with the Proc Freq procedure in the SAS (2017) package program. The results showed that the average age of the breeders was over 50 and most of the breeders were breeding buffaloes due to the habit from the past. In Çaycuma region, buffalo milk, buffalo yoghurt and buffalo meat used in sausage production have been identified as the most important income sources of enterprises.

Introduction

Animal husbandry is one of the oldest cultural activities of mankind. Long before the only cultural animal husbandry, people benefited from animals in different ways for many years in order to continue their lives (Mazoyer and Roudart, 2010; Şahin, 2015). Water buffalo breeding has become an important livestock activity in Turkey in recent years. In Asia, water buffalo have played an important role in overall social development, thanks to their contribution to the workforce required for milk, meat, leather and agricultural activities. Buffalo forms part of the farmers' goods, assets and business. Not only that, in some societies it is a reliable "living bank" and an easy "convertible source of money" to serve the urgent needs of the rural masses (Nanda and Nakao, 2003).

The name of the water buffalo (Water Buffalo) in English is due to its natural behavior in general, lying in wetlands, rolling and wandering (Soysal, 2009). The word buffalo, whose English equivalent is "Buffalo", is not generally described as a domestic animal, but as a part of zoos or natural life (Soysal, 2006).

Buffalo species are grouped as African wild buffalo and Asian buffalo. Domesticated and wild buffaloes, of which more than 70 different breeds are known, are divided into 2 groups as "River (River) Buffalo" and "Swamp Buffalo". The river buffalo group, which originated in India, is mainly bred to produce meat and milk, and they are combined productive breeds. Swamp buffaloes (Caraboa), on the other hand, are breeds that are not very suitable for milk production, are actually grown in China and Southeast Asia, and are used for labor as well as meat production (Atasever and Erdem, 2008; Sarıözkan, 2011). The water buffalo species in our country originated from the Mediterranean water buffalo in the subgroup of the river water buffalo and is called the "Anatolian Buffalo" (Soysal et al., 2005; Sarıözkan, 2011).

Anatolian water buffalo is grown for its three yields; meat, milk and labor. Most of the meat is used especially for sausage making. Research on milk production demonstrates that controlled rearing and feeding conditions can significantly improve performance. Buffalo milk, on the other hand, is used to produce yoghurt and to make a very popular traditional product, "lüle cream" (Soysal, 2014).

A large portion of the world's buffalo population (195 million heads) is raised in South and Southeast Asia, particularly in India (56.56%), Pakistan (17.17%) and China (12.56%). 121 826 heads of the buffalo population in Turkey are bred mostly in the Central Black Sea Region, especially in the provinces of Samsun, Tokat, Çorum and Amasya (25%). Lactation milk yield, lactation period, birth weight of calves and daily live weight gain during fattening of Anatolian buffalo were found to be 800 – 1000 kg, 200 – 250 days, 30 kg and 550 – 600 g, respectively (Uğurlu, 2017).

The aim of this study is to determine the current situation of water buffalo breeding in the Çaycuma region of Zonguldak province, and to identify the conditions for improving water buffalo breeding in this district, and to create an infrastructure for future studies.

Materials and Methods

The research material consists of water buffalo breeders and their breeding knowledge and practices in the villages of Çaycuma district of Zonguldak province. The people dealing with buffalo breeding in this region were determined and a face-to-face survey was conducted with all breeders who agreed to answer the questions. The aforementioned questionnaires formed the material of our research.

The research was conducted in three different areas as questionnaire, personal interview and data collection. In the process of communicating with the breeders, support was received from the veterinarians working in the Çaycuma District Directorate of Agriculture and Forestry, and personal interviews were made with the breeders in line with their guidance, the shelters were examined, the behavior of the buffaloes was observed and photographs were taken.

In the survey, 57 questions were asked to the breeders about the contribution of buffalo products to the family economy, the care, feeding and breeding of animals.

The data obtained as a result of the survey were analyzed with the Proc Freq method in the SAS (2017) package program.

Results and Discussion

The study was conducted by going to the enterprises of 36 farmers engaged in water buffalo breeding in the district. The age distribution of the surveyed breeders is given in Figure 1. Accordingly, it was determined that the breeders were between the ages of 25 and 83, the average age was 53, and 3%, 19%, 25%, 17% and 36% of them were 21-30, 31-40, 41-50, 51-60 and 61 years old and over. It has been determined that the buffalo breeders are predominantly 61 years old and over, while the breeders younger than 30 years old are very few.

The livelihoods of the breeders are given in Figure 2. 22% of the water buffalo breeders (8 people) in the district declared their livelihood as only animal husbandry, 3% (1 person) stated that they have additional income from plant breeding besides animal production, and 75% of them were working for official institutions or are private sector employees, besides animal husbandry.

When we look at roughage production (Figure 3), while 12 of 36 farmers do not produce forage crops, 24 of them produce barley, wheat, clover, corn, oats and vetch. Breeders produces; 9% corn only; 25% oats only; 9% wheat and corn; 13% wheat and oats; 8% clover and oats; 8% corn and oats; 8% corn and vetch; 4% wheat, clover and oats; 4% wheat, corn and oats; 8% corn, oats and vetch; 4% produces barley, wheat, clover and corn.

The state support status of breeders is given in Table 1 and Figure 4. Breeders benefits; 39% from only brood buffalo supports; 50% from brood buffalo and brood cattle supports; 3% from milk production, brood buffalo and brood cattle supports; 3% from brood buffalo, brood cattle and forage plant supports; 5% benefit from milk production, brood buffalo, brood cattle and fodder plant supports. All 36 breeders benefit from brood buffalo supports.

Membership status of breeders' associations is given in Table 2. While are members 17% of the breeders belong to the Buffalo Breeder Association; 8% to the Cattle Breeder Association; 5% to the Dairy Producers Association; 3% to the Buffalo Breeders and Dairy Producers Association; 3% of the Cattle Breeders and Dairy Producers Association, 64% are not members of any association. As it can be seen from the survey results, the majority of the water buffalo breeders did not become members of the Buffalo Breeders Association for various reasons, and some of the breeders who became members canceled their memberships.

Figure 5 shows the number of buffaloes by age. There were a total of 559 buffaloes in 36 breeders surveyed, of which 51% were cows, 21% female calf, 17% male calf, 6% bull and 5% heifer. As can be seen in Figure 5, cows constitute half of the water buffalo presence, and this is because they are used for a long time in breeding and most of the income in buffalo is derived from dairy products. When we evaluate the current buffalo situation according to their gender, 77% of them are females and 23% are males.

89% of the 36 breeders answered the age at first calving as 31-36 months, 11% as 37-43 months (Figure 6). No breeder expressed the options of 24-30 months

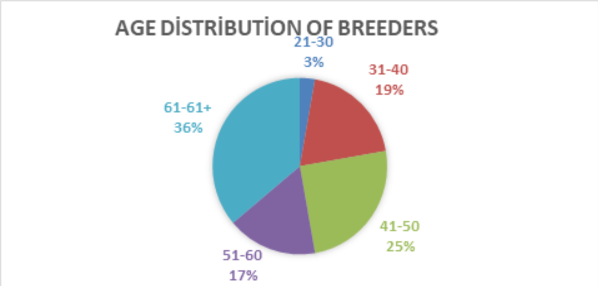

Figure 1. Age distribution graph of the breeders participating in the survey

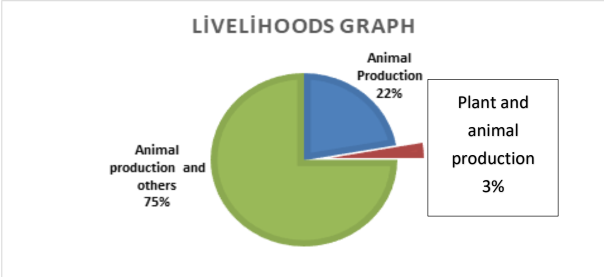

Figure 2. Livelihoods graph of breeders

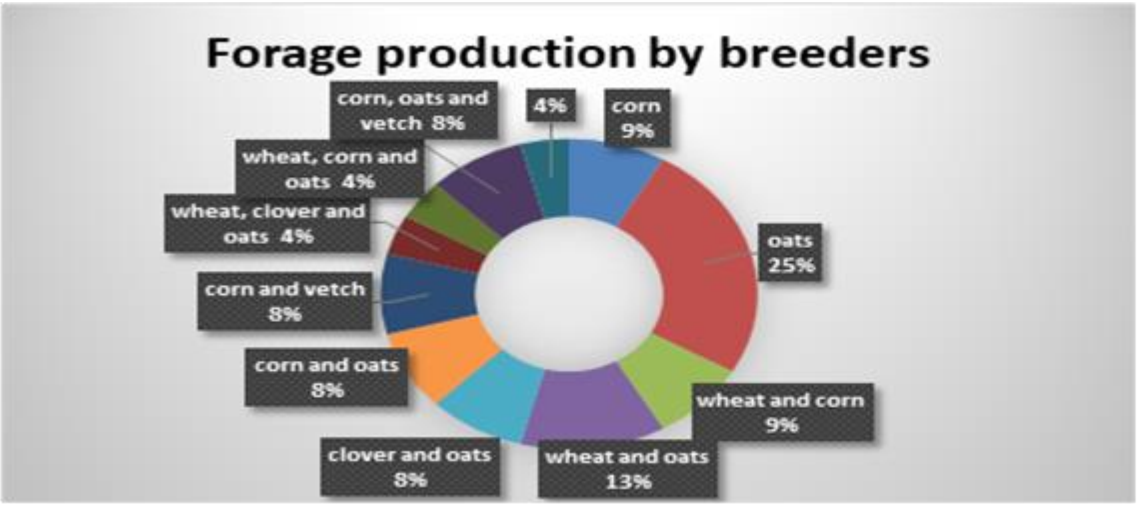

Figure 3. Age distribution graph of the breeders participating in the survey

Table 1. Number of breeders benefiting from government support

Support of Government	Plant Production	Milk Production	Buffalo	Cattle	Fodder Plant
Number of beneficiaries	0	3	36	22	3

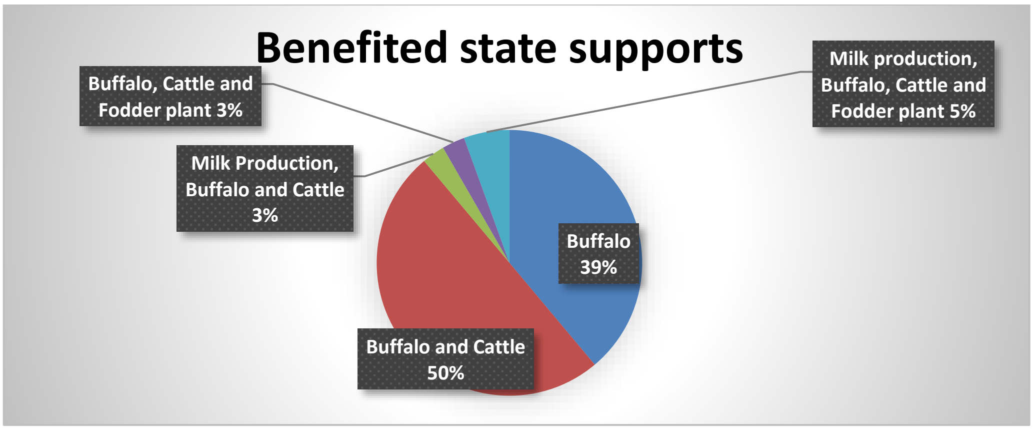

Figure 4. Benefited state supports

Table 2. Number of breeders who are members of breeder or producer associations

Breeders Association	Buffalo	Cattle	Milk producers	None	Breeders Association
Number of members	7	4	4	23	Number of members

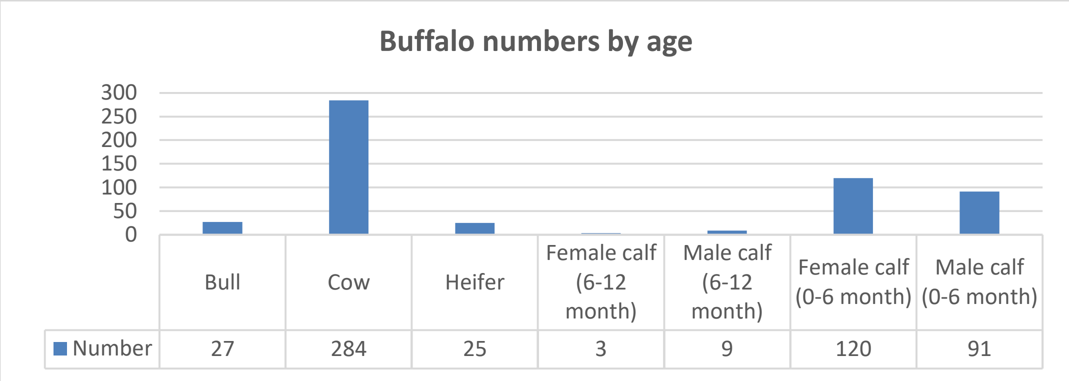

Figure 5 Number of buffaloes by sex and age

and 44-51 months. According to these results, the age of giving birth to the first claf is within the range specified in the literature (Küçükkebabçı and Aslan, 2002).

All of the breeders reported the interval between two calving as 11-15 months in buffaloes (Figure 7). Aksoy and Tekeli (1993) reported the average calving interval for Anatolian buffaloes as 462 days, and Şekerden (2002b) reported the ideal calving interval for water buffaloes as 13-14 months. In the survey, this period was reported as a maximum of 450 days. According to this result, the calving period, which is one of the factors affecting the fertility and therefore profitability, was considered suitable for extensive breeding in Çaycuma district.

For the maximum age of buffalo to stay in the herd, 55% of the breeders declared 11-15 years old, 31% 16-20 years old, 14% 6-10 years old, there was no breeder who declared the options 0-5 years old and older than 20 years. Based on the results, the breeders cull buffaloes after the age of 15, and none of them keeps buffaloes over 20 years old. They stated that the reason for this situation is that as the age progresses, the yield decreases and the animal weakens.

Figure 9 shows the graph of the age declarations at which buffaloes reach sexual maturity. According to this graph, 83% of breeders reported the age of sexual maturity as 23-24 months, 11% as 25 months and over, and 6% as 21-22 months. No breeder reported the 18-20 month option. According to the information given by the breeders, although sexual maturity can start after for them to reach sexual maturity. Aksoy and Tekeli (1993) reported that the breeding age for female buffaloes was determined as 20-24 months. This shows that the answers given by the breeders are similar.

When we examine Figure 10, it is seen that 97% of the surveyed breeders stated the survival rate in calves between 90-100% and 3% of them between 80-89%. It is possible to say that the survival rate of calves is over 95% throughout the district. Önal (2011) reported that the average survival rate for calves up to 6 months is 88%. When this value is compared with the survey results, it is seen that the viability rate of the calves in the region is high.

22% of the calves have a birth weight of 30 kg or less, 14% 31-35 kg, 36% 36-40 kg, 8% 41-45 kg, 3% 46-50 kg and 17% of them reported 50 kg and over (Figure 11). According to this information, we can say that the birth weight of the buffaloes reared in the district is mostly in the range of 30-40 kg. Aksel (2015) reported that the average birth weight of calves is 34 kg. When compared with the survey results, it is seen that the majority is close to this value.

Of the 36 breeders surveyed, 1 person reported that they gave colostrum to their calves for only 1 day, and all of the other 35 breeders for more than 3 days (Figure 12). There was no one who declared that she had been drinking for 2 and 3 days. As can be seen from these results, breeders are aware that colostrum is very important for calves.

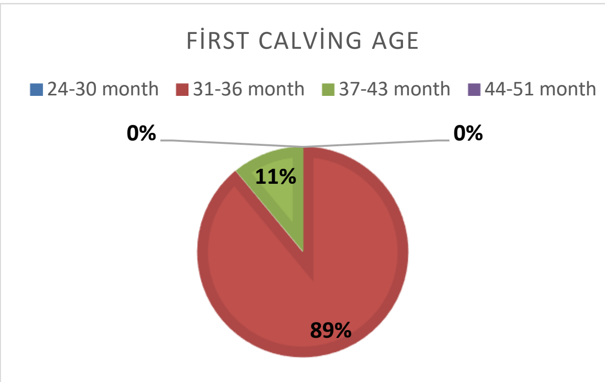

Figure 6. Age at first calving in buffaloes

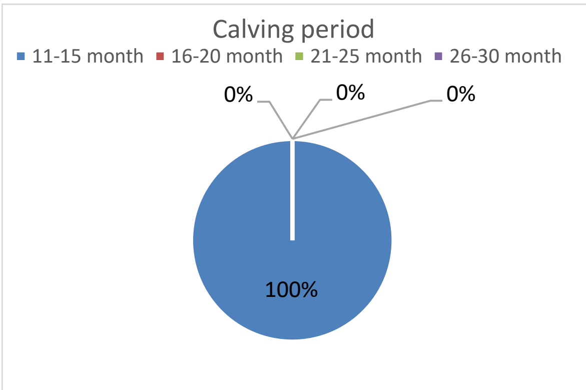

Figure 7. Calving interval of root buffaloes

The total pasture area in Çaycuma district is 673 hectares and they are pastures with low and medium productivity (Anonymous, 2019b). 86% of the breeders declared that the pastures they use are sufficient, 11% are insufficient, and 3% have declared that there is no pasture that they can use (Figure 13). According to these results, it is understood that the geographical conditions and pasture conditions of the villages in the district differ.

Black Sea climate prevails in the district. Precipitation usually occurs in the spring and autumn seasons. Snowfall is generally seen in December, January and February, but since it is not continuous, it does not prevent the animals from going to the pasture for a long time. As seen in Figure 14, 42% of the breeders stated that they could benefit from the pasture for 7-8 months, and 55% of them for 9 months or more. Only 3% stated that they do not take their animals to pasture. Yılmaz (2013) reported that the maximum duration of use of pasture in Afyon region is 8 months due to the weak resistance of water buffaloes to cold. As it can be understood from here, the Black Sea region is more advantageous than the regions in the interior in terms of the duration of using the rangeland.

In Table 3, information about the water source status of the rangelands in the district is given. 86% of the breeders declared that the pastures are near the stream, and 14% declared that they are by the artificial pond. It is important for water buffalo that most of the pastures are near the river. While the animals are grazing in the pasture, they can easily meet both their water needs and their cooling needs.

97% of the breeders reported that they followed the signs of estrus in buffaloes, and 3% did not (Figure 15). In the study conducted by Uslu (1970), it was reported that the animal should be given to the bull in the first estrus after birth, otherwise the next estrus can be seen 8-10 months later. It is important to follow estrus in buffaloes in terms of fertility.

When the breeders were asked about the daily average milk yield from buffaloes; 1 of them reported 2 liters, 18 of them 3 liters, 13 of them 4 liters, 3 of them 5 liters, 1 of them 7 liters of milk (Figure 16). However, these values are approximate or average values. For this reason, it is necessary to carry out measurement-based studies in order to determine the real milk yield in buffaloes.

64% of surveyed breeders reported that the lactation period was between 180 – 210 days and 36% between 210 – 270 days (Figure 17). Uğurlu (2017) reported the lactation period of Anatolian buffalo as 200-250 days. According to these values, it is possible to say that the lactation periods in the region are generally of medium length.

The breeders were asked about the products they earn from the buffalo and 9 of the 36 breeders were only yoghurt; 22 of them are milk and yogurt; 2 of them are milk, yogurt and butter; 1 of them is milk, yogurt and cream; 1 of them milk, yogurt and meat; 1 of which stated as milk, yogurt, butter and cream. As can be seen in Table 4, the most profitable buffalo product in the region is yogurt in the first place and milk in the second. Butter and cream are produced by some breeders when there is customer demand.

According to Table 5, 33 of the 36 breeders surveyed stated that they sold their butcher buffaloes alive to the buyers who came to the village, 4 of them sold them alive to the slaughterhouse, 1 of them cut themselves and sold them as meat to the villagers. No breeder has said that she cuts her own animal and meets her meat needs.

The breeders were asked what they thought about the water buffalo breeding in Çaycuma, and 18 people stated that the number of animals is not given importance, so the number of animals may decrease even more, 1 person stated that the number of those dealing with water buffalo may increase due to the support given in recent years, and 17 people stated that the supports and investments given to water buffalo are insufficient (Table 6). There were no breeders who stated that buffalo breeding would develop in the district.

When asked which suggestions would be appropriate for the development of Çaycuma and Turkish buffalo breeding, 56% of the breeders stated that state subsidies should be increased, 24% stated that the breeding works should be accelerated urgently, and 20% stated that the number of buffaloes should be increased (Table 7). It is necessary to increase the state subsidies every year and to increase the types of support.

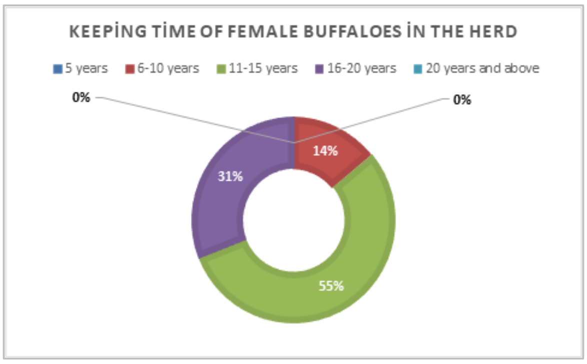

Figure 8. Keeping time of female buffaloes in the herd

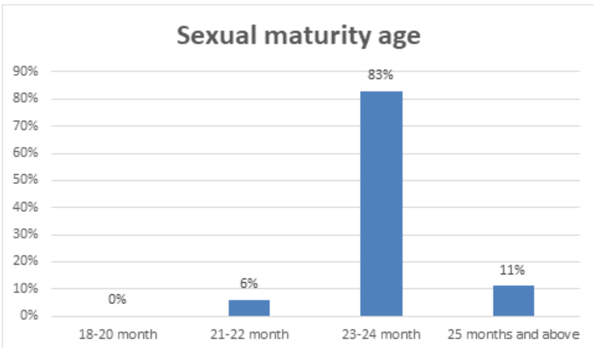

Figure 9. Age of sexual maturity in buffaloes

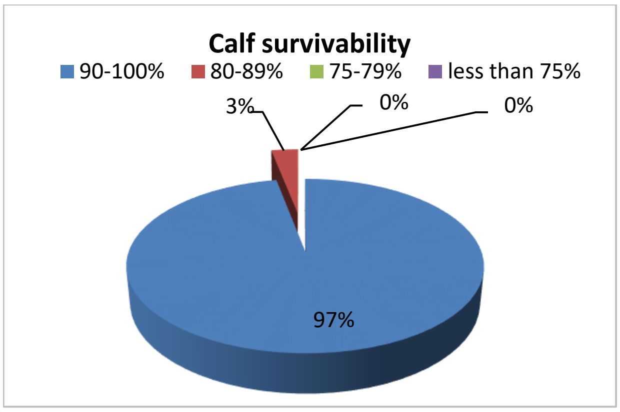

Figure 10. Survival rate in calves

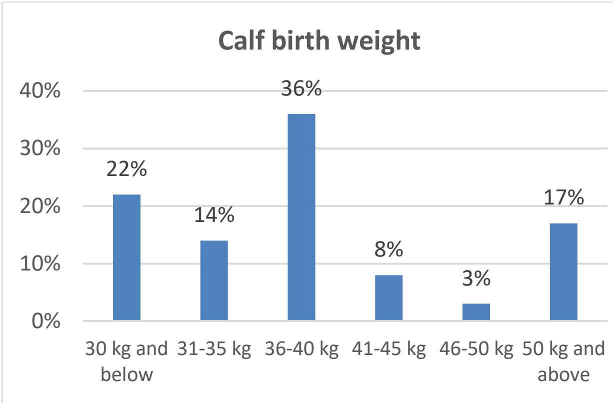

Figure 11. Birth weight of calves

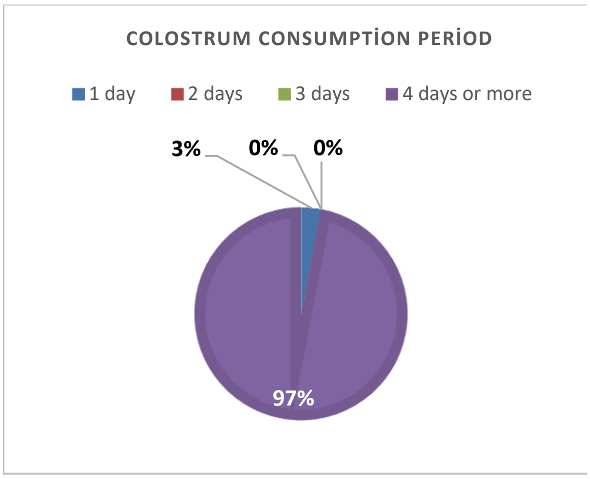

Figure 12. The colostrum consumption period of calves

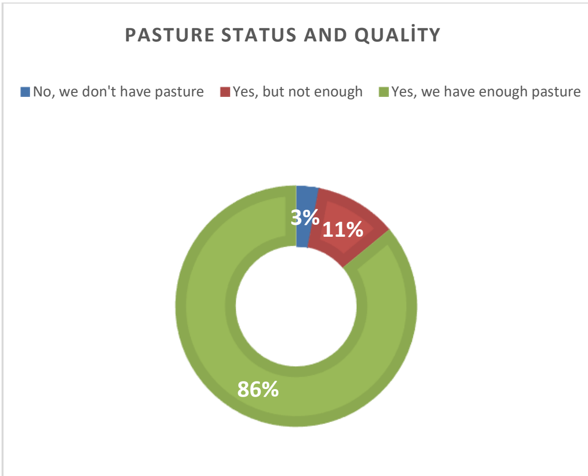

Figure 13. Pasture status and quality

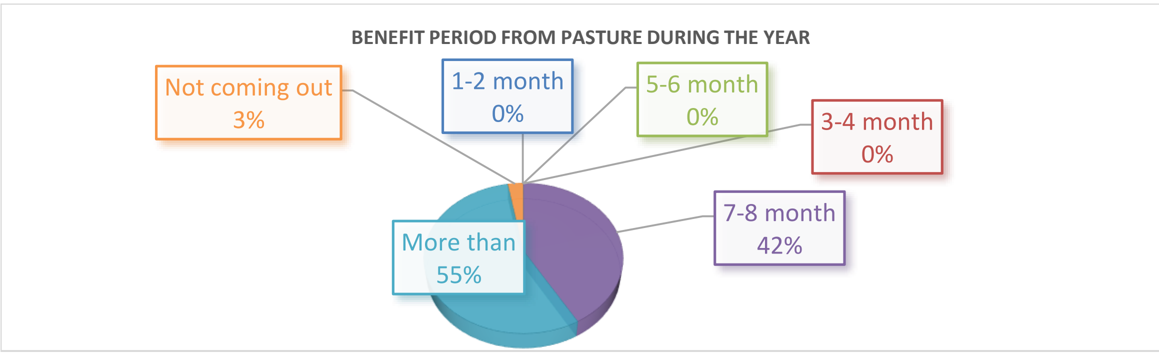

Figure 14. Benefiting period of pasture during the year

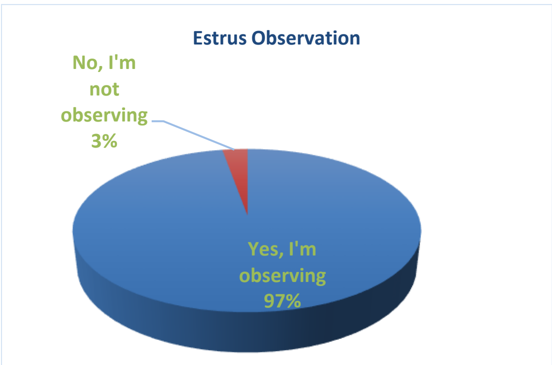

Figure 15. Observing estrus

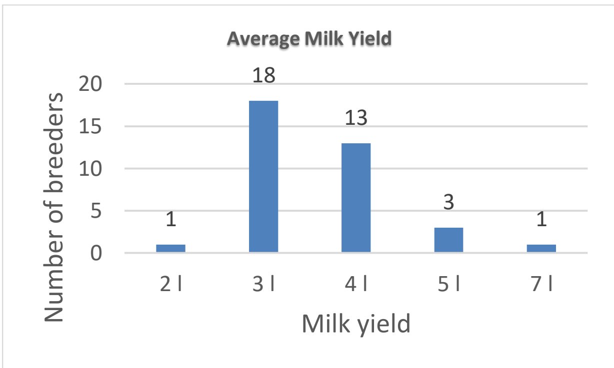

Figure 16. Average daily milk yield in buffaloes (l)

Table 3. The situation of water resources in the village pasture

Number of Breeders Who Say We Have No Water Source		**0**
Number of Breeders Saying We Have a Water Source	Lakeside	0
	Stream Edge	31
	Artificial Pond Edge	5

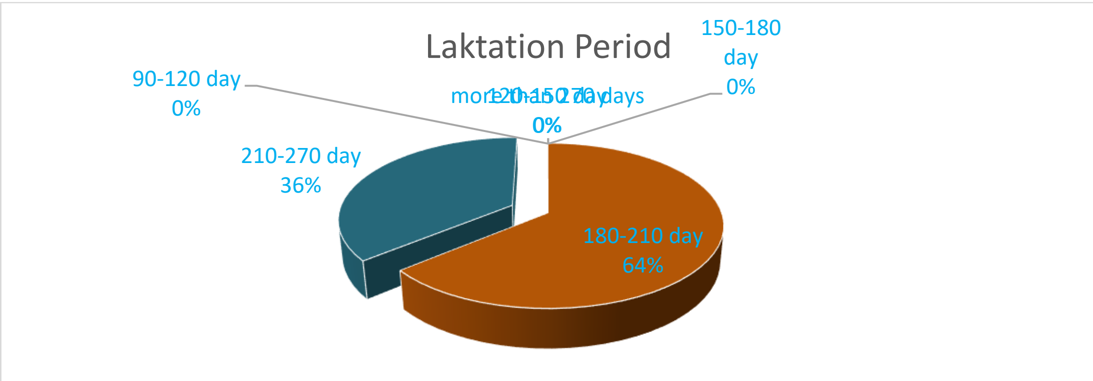

Figure 15. Observing estrus

Table 4. The products that breeders earn

İncome generating products	Number of breeders
Yogurt	9
Milk/Yogurt	22
Milk/Yogurt/Butter	2
Milk/Yogurt/Cream	1
Milk/Yogurt/Meat	1
Milk/Yogurt/Butter/Cream	1

Table 5. Evaluation of butchery buffaloes

Evaluation of butchery buffaloes	Number of breeders
I take a nap myself and get my meat needs	0
I take a nap myself and sell it in the village	1
I sell live to the slaughterhouse	4
I sell live to buyers	33

Table 6. Opinions of breeders about the future of water buffalo breeding in Çaycuma region

The future of water buffalo in the Çaycuma region	Number of breeders
The number of animals may decrease gradually as buffalo breeding is not given importance.	18
The number of buffaloes will increase due to the support given in recent years.	1
I think that the support and investments for buffalo are insufficient.	17
I think that buffalo breeding in our district will develop.	0

Table 7. Recommendations for buffalo breeding in the district and in our country

Recommendations for the development of buffalo breeding	Number of breeders
Animal production work should be accelerated urgently	10
The presence of buffalo should be increased	8
State subsidies should be increased	23

Conclusion

The age profile of breeders in the district is mostly over 61 years old and the average age is determined as 53. The number of breeders who make a living from only animal husbandry is very few. Mainly grown forage crops are oats, wheat, clover and corn. Mainly brood buffalo and brood cattle state support is used. More than half of the breeders are not members of any breeder association.

Buffaloes are usually taken out to pasture more than 8 months a year. There is no pasture without a water source and there is usually a stream in the pastures. The most used roughage was determined as straw and dry clover. Grain feeds are not used in the supply of mixed feed and commercial feeds are preferred.

According to the breeder's notifications, the average daily milk yield of the buffaloes in the region is around 3.5 liters. Lactation period is generally between 180-210 days. Milk from buffaloes; It is evaluated as yoghurt or raw milk and cream is rarely made according to demand. For this reason, the products that provide the main income in buffalo farming are yogurt and raw milk.

The breeders are of the opinion that the number of animals will decrease even more since water buffalo breeding is not given importance, that the support given to water buffalo is insufficient, therefore, if the current conditions continue, water buffalo breeding in the region will be in danger of not developing.

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Author contributions

First Author: Investigation, Data Curation, Visualization and Writing;

Second Author: Supervision, Writing - review and editing.

Conflict of Interest

The author declare no conflicts of interest.

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LIVESTOCK STUDIES

RESEARCH PAPER

Determination of the Effects of Grape Pomace Addition to Sorghum Sudan Grass on Silage Quality

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Abstract

The aim of this study was to determine the silage quality and *in situ* degradability of silages prepared with addition of grape pomace into variety of sorghum-sudan grass as rapid fermentable carbohydrate source. Grape pomace obtained at the region was ensiled with sorghum- sudan grass grown at Keskin Fodder Plants Production and Processing Facility at same period at the levels of 0, 10, 20 and 40%. Glass jars (1L) were used for ensiling of silages. Four silage samples were prepared for each treatment groups. After 45 days of ensiling, silage samples were opened and pH, organic acid, nutrient contents, and *in situ* degradation levels were determined. Among silage fermentation parameters, pH and volatile fatty acid concentrations did not differ among silage prepared from different sorghum-sudan grass varieties (P>0.05), grape pomace significantly increased the pH of sorghum-sudan grass silages and decreased lactic acid concentrations of sorghum-sudan grass silages (P<0.05). Nutrient contents, except CP content, significantly differed between sorghum-sudan grass varieties, addition of grape pomace into sorghum-sudan grass significantly alter the nutrient contents of silages (P<0.05). While in situ OM, NDF and ADF degradabilities were similar between sorghum-sudan grass varieties, addition of grape pomace significantly decreased OM degradability in both sorghum-sudan grass varieties (P<0.05). In conclusion, addition of grape pomace into sorghum-sudan grass up to 40% had some negative effects on silage quality, but it was taught that grape pomace can be utilized as alternatve feedstuffs for ruminants by adding sorghum-sudan grass up to 20%.

Introduction

One of the biggest problems of the livestock sector is insufficient production of roughage, in terms of both quantity and quality, which should be included in the rations of ruminant animals (Bingöl *et al.*,2010). In order to minimize the quality roughage needs for livestock enterprises, forage crop production areas should be increased, meadow and pasture qualities should be improved, especially cheap and alternative roughage sources (pulp) should be brought into animal production and quality roughage production techniques should be transferred to breeders. If abondant and cheap high quality orage is avalable, then, an economical animal breeding can be possible (Alçiçek *et al.*, 2010). It has been accepted by the world as a result of researches the importance of roughage, especially in dairy cattle. Many wet by-products are ensiled and the animals are constantly fed with fresh feed over a whole year (Faostat, 2011).

Increasing the human population in the world increases the need for animal products. The increase in the number of animals and the inadequacy of forage crops production have led scientists to alternative roughage. In Turkey 3.650.000 tons of grapes are produced annually, and 423,527 tons of this has been utilized for wine production (TÜİK, 2015). This wine type grape yields 105,882 tons of pomace per year. This figure corresponds to 25% of the wine type grapes (Kılıç and Abdiwali, 2016).

Since the grape pomace deteriorates rapidly due to its high water content, it should either be consumed in a short time or dried so that the water content decreases to 10% (Özdüven *et al.*, 2005). Otherwise, it is not possible to preserve it. It has been reported that the addition of grape pomace in alfalfa silages at the rate of 4% - 20%, increased both silage quality and *in vitro* digestibility, in parallel with the increases in silage (Canbolat *et al.*, 2010).

Although the remaining grape pomace, which is generally byproduct of wine and vinegar production, is offered to animal consumption as fresh by livestock enterprises, it is not possible to store it for a long time because of its high water content. Therefore, the effects on quality and *in situ* degradability of silages prepared by adding varying amounts of grape pomace to two different sorghum-sudan grass hybrids grown in Kırıkkale province were evaluated in the study.

In this study, it was aimed to determine the effects of adding at varying rates of grape pomace to two different sorghum-sudan grass hybrids on silage quality, nutrient composition and *in situ* degradability of silages.

Materials and Methods

In this study, the effects on silage quality and *in situ* degradability were tried to be determined by adding varying amounts of grape pomace to two different sorghum-sudan grass hybrids grown in Kırıkkale province. For this purpose, grape pomace samples were collected from plants producing fruit juice, vinegar, molasses and wine in the region during 2014 and 2015 fruit harvest periods. Sorghum-sudan grasses were produced in Keskin Fodder Plants Production and Processing Facility in the same period. It was ensiled by mixing sorghum-sudan grass with 0% (control), 10, 20 and 40% grape pomace. One liter jars were used for ensiling the silages. Each experimental group was prepared as 4 replications. After the jars were tightened as much as possible by hand, their lids were tightly closed and pierced with a nail. The silages prepared in jars were turned upside down and left to mature in a dark and cool room. A few days after the silage water removal, the holes opened with nails were closed with a tape and left to mature. Silages opened after 45 days of maturation and pH, volatile fatty acid, nutrient contents and *in situ* degradability were determined. After the silages were dried first in air and then in an oven, other nutrient analyzes were made.

The pH and volatile fatty acid analyzes of the silages were determined from the filtrates obtained from the silages. For this purpose, after adding 100 ml of distilled water on 25 g of silage sample, this mixture was thoroughly homogenized with the help of a blender. The pH of the silage was determined by measuring with a pH meter (Polan *et al.*, 1998). The silage liquid obtained by filtering through filter paper (Whatman) was stored at -18ºC until the organic acid analysis.

Three rumen cannulated Holstein dairy cows were used to determine the *in situ* degradability of the silages. During the experiment, cows were fed twice a day with alfalfa grass and barley straw as roughage and barley and wheat flakes mixture as concentrated feed. During the trial period, the animals were fed with a ration consisting of 60% roughage (50% alfalfa hay and 50% barley straw) and 40% grain feed (50% barley and 50% wheat flakes). Animals were consumed this ration for a total of 16 days, of 14 days of adaptation and 2 days of sample collection. Clean water and vitamin-mineral blocks were kept in front of the animals at all times.

The feed samples incubated were ground to a particle size of 2 mm after drying. The feed samples were weighed 3-4 g in two parallel for each animal and for each hour and put into 10 x 5 cm dacron pouches with a 40 µ pore size. After the mouths of the pouches were tightly tied with rubber bands, they were placed in nylon nets with a size of 40x20 cm, with a pore size of 0.3 cm, in which weight was placed in order to keep them in the ventral part of the rumen.

The nets are placed in the ventral cavity of the rumen of the cows. The prepared nylon bags were left in the rumen for 0, 2, 4, 8, 12, 24 and 48 hours. At the end of each incubation period, the pouches were removed from the rumen and the contaminated feed particles were removed by washing with pressurized cold water to prevent microbial activity. Then, the pouches were washed under running water until the color of the water became clear (approximately 15 minutes) dried in an oven at 65ºC for 24 hours (Çetinkaya, 1992), and their weights were recorded. The dry matter (DM), organic matter (OM) neutral detergent fiber (NDF) and acid detergen fiber (ADF) contents of the remaining feed residues in the pouches were determined and the OM, NDF and ADF degradability were calculated according to the following formula;

nutrient degradability = $a + b^{(1-e-ct)}$ (Orskov 1985).

The DM, crude ash (CA), OM, and crude protein (CP) contents of the feed samples used in the experiment were determined according to the AOAC (1990), NDF according to Van Soest and Robertson (1979), ADF according to Goering. and Van Soest (1970).

In the filtrate obtained from silage samples, organic acids (lactic acid, acetic acid, propionic acid and butyric acid) analyses were run according to the method reported by Leventini *et al*. (1990) at the gas chromatography (Shimadzu GC14B). Ammonia determination was made by the distillation method specified by Filya (2003).

All data obtained in the study were subjected to two-way analysis of variance SAS, (1995). First, the statistical difference between sorghum cultivars was determined and given as P value in the tables. Then, the Duncan-t test was used to determine the differences due to grape pomace use in both sorghum varieties (Steel and Torie, 1980).

Results and Discussion

The nutrient contents of the grape pomace used are given in Table 1. When the table is examined, it is seen that white and black grape pomace have similar nutrient content, but black grape pomace has a little bit higher of crude cellulose (CC), NDF and ADF content while the HP level is slightly lower.

Ensminger *et al*. (1990) reported that the nutritional content of grape pomace; CC (30.9%), EE (8.4%), CP (13.4%), nitrogen free substance (39.0%), OM (91.7%), CA (8.3%), NDF (53.2%), ADF (44.4%) and ADL(35.2%). Kılıç and Abdiwali (2016) determined that CA, EE, CC, NDF, ADF and CP values of dried grape pomace were 8.2%, 4.9%, 19.80%, 49.60%, 38.30% and 12.50%, respectively. The crude protein, ADF, NDF, EE and CC contents were similar to the studies mentioned above, while CA values were lower than the studies. Winkler *et al*. (2015), the CA (6.8-3.3%) reported for the pomace of white and red grape varieties was similar with the current study. The differences in the nutrient content of grapes between the studies are probably due to the grape variety of the pomace is obtained, the different applications, the differences in the stalk and seed ratios of the pulp, and the differences in the foreign matter contents such as dust and soil (Kılıç and Abdiwali, 2016).

Nutrient contents of sorghum-sudan grass silages prepared with grape pomace in different proportions are given in Table 2. It was observed that the DM value of the silages prepared from sorghum-sudan crops varied between 25.83-30.75% and there was a statistical difference between the varieties ($P<0.05$). In this study, it was determined that the DM levels were around the ideal DM levels reported by Ergün *et al.* (2001) for silage. The dry matter levels of the silages were affected by the DM levels of the plant species rather than the grape pomace used. The added grape pomace level caused a decrease in the DM level of the Sugar Graze II (SG II) variety containing only 40% of the silage. The reason for this decrease is not understood.

The OM levels of the silages prepared by adding different levels of grape pomace to sorghum-sudan grass were in the range of 90.41-93.23% and similar between sorghum-sudan grass varieties ($P>0.05$). Çiğdem and Uzun (2006) have reported that CA ratios of sorghum x sudan grass hybrid varieties were in the range of 7.84%-8.64%. Also, Salman and Budak (2015) determined the CA values of sorghum x sudan grass hybrids between 6.42% and 9.53% in a study conducted in Ödemiş and Bayındır districts. CA values of the both studies was similar with that of the current study.

Addition of grape pomace to silages did not significantly affect the OM level of sorghum-sudan grass silages in general ($P>0.05$). The CA content of the grape pomace used in the study was at the level of 6% and it is thought that it does not cause any

Table 1. Nutrient contents of grape pomace used in the study (DM%)

	White Grape Pomace	**Black Grape Pomace**
Dry matter	31,05	32,07
Crude ash	5,50	6,35
Organic matter	94,50	93,65
Ether extract	6,75	6,25
Crude cellulose	26,59	27,67
Neutral detergent fiber	43,73	45,93
Acid detergent fiber	34,86	37,57
Crude protein	12,42	11,98

Table 2. Nutrient contents of silages obtained in the study.

	Sorghum Sudan Grass SG II				Sorghum Sudan Grass GAR				
	0	10	20	40	0	10	20	40	P
DM,%	28,53±1,23^{1}	28,17±1,82^{1}	29,72±1,08^{1}	25,83±1,62^{2}	32,75±0,23	29,11±1,43	31,76±0,87	31,87±0,48	0.05
CA,DM%	6,99±0,56	6,77±0,51	7,29±0,25	7,10±0,34	7,37±0,54^{b}	7,23±0,65^{b}	7,51±0,71^{b}	9,59±0,86^{a}	0.45
OM,DM%	93,01±0,56	93,23±0,51	92,21±0,25	92,90±0,34	92,63±0,54^{a}	92,77±0,65^{a}	92,49±0,71^{a}	90,41±0,86^{b}	0.45
NDF,DM%	60,94±0,44	58,80±0,41	61,62±0,47	62,86±0,33	49,68±2,02^{b}	55,40±0,80^{a}	56,14±1,07^{a}	55,13±1,40^{a}	0.05
ADF,DM%	28,63±0,15^{4}	30,09±0,06^{34}	34,47±0,9^{2}	42,77±0,55^{1}	21,55±1,26^{c}	27,06±1,71^{b}	27,24±2,65^{b}	33,96±1,32^{a}	0.05
CP,DM%	10,63±0,11^{2}	10,62±0,86^{2}	13,64±0,85^{1}	11,19±0,86^{2}	10,71±0,72	11,13±1,01	12,57±0,77	11,71±0,59	0.65

SG II: Sugar Graze II; GAR: Gardavan; DM: Dry matter; CA: crude ash; OM: organic matter; NDF: neutral detergent fiber; ADF: acid detergent fiber; CP: crude protein.
a,b,c: Different letters on the same rows indicate statistical difference for Sorghum Sudan Grass GAR (P<0.05).
1, 2, 3: Different numbers on the same rows indicate statistical difference for Sorghum Sudan Grass SG II (P<0.05).

change in sorghum-sudan grass, since CA level is close to that of sorghum-sudan grasses.

It is seen that NDF and ADF contents of sorghum-sudan grass silages prepared by mixing with grape pomace in varying proportions were between 49.68-62.86% and 21.55-42.77%, respectively. While there was a significant difference (P<0.05) in terms of NDF and ADF values of sorghum-sudangrass variety, it was determined that the added grape pomace only was increased the NDF values of Gardavan (GAR) sorghum-sudan cultivars statistically (P=0.05). Grape pomace added to plant crops statistically increased the ADF values of both sorghum-sudan grass silages. It has been reported that the NDF values of the silages prepared from different sorghum-sudan grass varieties are in the range of 68.45-71.53%, the ADF values are in the range of 38.48-43.69%, and there are significant differences between the varieties (Akdeniz et al., 2005). Also, Karadağ and Özkurt (2014) reported that while NDF values of different sorghum sudan varieties were between 62.01% and 62.66% and ADF values were between 39.14% and 40.86%. It is seen that NDF and ADF values of sorghum-sudan grass silages obtained in this study are similar with theese studies. It is thought that the reason of the differences between the varieties is due to the fact that the NDF and ADF values of the grape pomace are higher than the NDF and ADF values of the GAR variety.

CP values of sorghum-sudan silages in this study were between 10.62-13.64%. In a study conducted by Çiğdem and Uzun (2006), the CP values of sorghum x sudan grass varieties ranged from 6.07% (Jumbo variety) to 10.16% (El Rey variety). Karadağ and Özkurt (2014) determined the CP ratio of silage sorghum as a second crop to be 9.45-10.99%. The highest CP values obtained in this studies are similar to the CP values of sorghum-sudan grass silages in mentioned study. The nutrient content of plants differs depending on many factors such as variety, soil structure, harvest period and fertilizer dose used.

Although the effect of grape pomace on the CP values of the silages was not statistically significant (P>0.05), it was observed that the addition of grape pomace to sorghum-sudan silages caused an increase in the CP values of the silages in general.

The parameters regarding to the fermentation quality of the silages are given in Table 3. The pH values of the silages prepared with sorghum-sudan grass were in the range of 3.89-4.87. Grape pomace increased silage pH linearly. Arslan and Çakmakçı (2011) determined that the pH of

Table 3. Fermentation parameters of silages obtained in the study.

	Sorghum Sudan Grass SG II				Sorghum Sudan Grass GAR				
	0	10	20	40	0	10	20	40	P
pH	4,15±0,05^{2}	4,19±0,04^{2}	4,21±0,07^{2}	4,61±0,13^{1}	3,89±0,02^{c}	4,01±0,03^{b}	4,03±0,02^{b}	4,87±0,29^{a}	0.48
Laktic acid,DM%	3,14±0,315^{1}	2,21±0,171^{2}	2,68±0,305^{12}	0,70±0,075^{3}	2,34±0,294	2,60±0,451	1,92±0,074	1,87±0,275	0.38
Asetic acid,DM%	0,63±0,123^{1}	0,65±0,050^{1}	0,66±0,018^{1}	0,38±0,025^{2}	0,24±0,025	0,43±0,131	0,40±0,138	0,61±0,251	0.42
Propionic acid,DM%	0	0	0	0	0	0	0	0	1.00
Bütiric acid,DM%	0	0	0	0	0	0	0	0	1.00
Ammonia nitrogen,DM%	0,57±0,071	0,53±0,021	0,48±0,105	0,60±0,103	0,46±0,051	0,41±0,075	0,48±0,125	0,50±0,085	0.86

SG II: Sugar Graze II; GAR: Gardavan;
a,b,c: Different letters on the same rows indicate statistical difference for Sorghum Sudan Grass GAR (P<0.05).
1, 2, 3: Different numbers on the same rows indicate statistical difference for Sorghum Sudan Grass SG II (P<0.05).

Table 4. The OM degradation values of silages, DM%.

H	Sorghum Sudan Grass SG II				Sorghum Sudan Grass GAR				
	0	10	20	40	0	10	20	40	P
0	10,94±1.10	7,94±0.89	10,21±0.78	6,59±0.65	13,36±1.06^{b}	18,88±1.36^{a}	19,97±1.23^{a}	7,56±0.78^{b}	0.43
2	11,29±0.87^{2}	11,71±1.05^{2}	12,06±1.76^{12}	15,02±1.83^{1}	10,02±1.97^{b}	11,70±1.21^{b}	18,4±1.43^{a}	10,90±1.07^{b}	0.26
4	14,31±1.34^{1}	11,70±1.45^{12}	15,72±1.23^{1}	9,30±1.07^{2}	15,63±1.31^{a}	14,22±1.67^{a}	10,65±1.03^{ab}	16,94±1.87^{a}	0.34
8	16,61±1.32^{23}	12,58±1.43^{3}	21,26±1.83^{1}	17,64±1.11^{12}	20,73±1.34^{b}	18,62±1.57^{b}	23,71±1.96^{a}	16,58±121^{c}	0,08
12	26,52±1.56^{1}	19,12±1.65^{12}	17,24±1.75^{2}	19,91±0.861^{2}	22,07±1.55^{a}	20,46±1.11^{a}	29,28±2.12^{a}	25,47±2.54^{a}	0.67
24	38,15±2.03^{1}	39,99±1.89^{1}	40,09±1.54^{1}	32,05±.21^{2}	46,68±2.03^{a}	40,63±2.11^{b}	33,86±2.05^{c}	25,74±1.78^{d}	0.45
48	61,09±2.86^{1}	56,15±2.32^{2}	56,28±2.05^{2}	47,63±1.25^{3}	65,90±2.89^{a}	62,89±2.06^{ab}	61,70±2.45^{ab}	58,78±2.01^{b}	0.25

SG II: Sugar Graze II; GAR: Gardavan;
a,b,c: Different letters on the same rows indicate statistical difference for Sorghum Sudan Grass GAR (P<0.05).
1, 2, 3: Different numbers on the same rows indicate statistical difference for Sorghum Sudan Grass SG II (P<0.05).

sorghum-sudan grass silage without any additives was 3.90. Also, the pH values reported by Keskin *et al*. (2005) for sorghum-sudan grass silage are similar to that of the current study. The silage pH values of the current study were around or slightly above 3.8-4.2 which reported by Ergün *et al.* (2001) that the optimal pH range for silages, but it is in the acceptable range for a good silage. This shows that a good fermentation is formed in silages.

Organic acid levels of silages are presented in Table 3. While sorghum-sudan grass silages are mostly rich in lactic acid, it is seen that grape pomace addition to silages reduce lactic acid levels (P<0.05). While lactic acid levels in sorghum-sudan grass were 0.70-3.14% DM in SG II veriety, these values were 1.87-2.34% DM in GAR variety. The addition of grape pomace in both silages affected the lactic acid profile of the silages, but this effect was more pronounced in the SG II variety (P<0.05). In parallel with the increase in grape pomace level, lactic acid was decreased in both sorghum-sudan grass silages. This change was also reflected in silage pH. It has been determined that sorghum-sudan grass silages contain serious levels of acetic acid. This indicates that these silages are heterofermentative type silages. The amount and profile of organic acid released as a result of fermentation by lactic acid bacteria in silages are related to the sugar content, moisture and buffering capacity of the silage product (Rotz and Muck, 1994). Buffering capacity is lowest among crops in maize, medium in meadow grasses and highest in legumes. For this reason, it is difficult to lower the pH of silage in legumes, and Clostridia, an anarobic bacteria, are vital in these type of siages. These bacteria can ferment sugar, lactic acid and amino acids, resulting in the formation of butyric acid and ammonia-N (Rotz and Muck, 1994). Butyric acid, which is a sign of deterioration or poor quality silage, was not found in the silages of the current study. If not this is a sign that there is no serious problem in the conservation of silages (Ergun *et al*., 2001).

Ammonia-N levels in sorghum-sudan grass silages were 0.41-0.60, % DM and similar (P>0.05). Silage ammonia-N level is an expression of the water-soluble CP level in silages. It is seen that the ammonia levels of the silages change in parallel with the CP

Table 5. The NDF degradation values of silages, DM%.

H	Sorghum Sudan Grass RE				Sorghum Sudan Grass ME				
	0	10	20	40	0	10	20	40	P
0	0,21±1.83	0,73±1.03	3,81±1.23	4,64±1.43	1,81±1.42	0,41±1.71	2,06±0.76	1,83±1.02	0,80
2	1,41±0.89^{2}	0,32±1.25^{2}	2,08±1.03^{2}	7,85±2.78^{1}	0,0±0.56^{b}	1,70±1.01^{b}	5,15±2.26^{a}	3,21±1.83^{ab}	0.26
4	3,63±1.52^{2}	0,94±0.78^{2}	10,04±2.23^{1}	13,22±2,32^{1}	1,31±0.84^{b}	1,00±0.45^{b}	6,58±2.41^{ab}	11,99±3,31^{a}	0.34
8	3,43±2.36^{2}	1,42±1.78^{2}	19,32±3.81^{1}	24,64±4.11^{1}	0,77±0.36^{b}	9,76±2.58^{a}	10,22±2.06^{a}	13,74±1.78^{a}	0.43
12	16,41±2.65^{a}	10,88±3.3^{a}	25,08±3.26^{1}	32,18±3.21^{2}	5,15±1.65^{b}	10,43±3.27^{a}	16,85±3.11^{a}	17,76±2.87^{a}	0.27
24	33,86±3.95^{2}	31,99±3.89^{2}	36,11±1.54^{12}	41,0±3.86^{1}	31,46±3.13^{a}	29,50±3.41^{a}	17,86±3.05^{b}	18,59±3.54^{b}	0.08
48	58,48±4.86^{1}	50,20±3.12^{12}	51,28±.45^{12}	45,20±3.23^{2}	54,43±2.98^{a}	49,20±3.16^{b}	53,20±2.45^{ab}	48,43±3.12^{b}	0.35

SG II: Sugar Graze II; GAR: Gardavan;
a,b,c: Different letters on the same rows indicate statistical difference for Sorghum Sudan Grass GAR (P<0.05).
1, 2, 3: Different numbers on the same rows indicate statistical difference for Sorghum Sudan Grass SG II (P<0.05).

Table 6. The ADF degradation values of silages, DM%.

H	Sorghum Sudan Grass RE				Sorghum Sudan Grass ME				
	0	10	20	40	0	10	20	40	P
0	1,41±0.92	0,32±0.93	1,31±1.06	0,91±0.86	3,48±1.52	1,12±1.27	1,95±0.96	0,86±0.78	0,58
2	0,69±0.87[2]	0,85±1.05[2]	4,83±1.78[12]	11,32±2.97[1]	1,78±0.97	1,77±1.01	1,14±1.03	3,64±1.87	0.26
4	0,55±0.74[2]	0,94±0.89[2]	5,27±1.58[12]	10,67±2.65[1]	3,3±1.21[b]	0,24±0.67[b]	1,94±1.37[b]	14,05±2.78[a]	0.34
8	1,28±1.10[2]	5,13±1.87[2]	17,97±2.12[1]	20,60±2.88[1]	1,21±1.06[b]	1,31±1.11[b]	4,75±1.03[b]	18,89±2.21[a]	0.23
12	12,84±1.76[2]	9,29±2.15[2]	25,08±2.89[1]	32,05±3.21[1]	13,09±2.15[b]	6,89±1.96[b]	13,75±3.12[b]	30,01±3.58[a]	0.67
24	33,86±2.53[2]	31,24±2.59[2]	36,41±2.51[12]	41,10±3.41[1]	21,57±2.73[b]	26,44±2.26[b]	15,50±2.15[c]	31,12±3.87[a]	0.05
48	60,73±2.98[1]	44,52±3.13[2]	48,04±3.25[2]	43,72±4.25[2]	41,08±3.59[b]	41,73±3.61[b]	45,78±3.24[ab]	47,4±3.11[a]	0.25

SG II: Sugar Graze II; GAR: Gardavan;
a,b,c: Different letters on the same rows indicate statistical difference for Sorghum Sudan Grass GAR (P<0.05).
1, 2, 3: Different numbers on the same rows indicate statistical difference for Sorghum Sudan Grass SG II (P<0.05).

content. Grape pulp did not cause a significant change in ammonia-N content in sorghum-sudan grass silages. The change in the CP values of the silages was also reflected in the ammonia levels of the silages.

The *in situ* degradation values of OM, NDF and ADF of sorghum-sudan grass silages are given in Tables 4, 5, 6. Although the degradation values of OM, NDF and ADF obtained after 48 hours of rumen incubation for sorghum-sudan grass variety differ in numbers, they are statistically similar (P>0.05). While OM degradation in silages prepared with sorghum-sudan grass varied between 58.78-65.90% in GAR varieties, these values were in the range of 47.63-61.09% in SG II variety after 48 hours. Addition of grape pomace to significantly reduced OM degradation in both varieties, and the lowest OM degradations were seen in groups containing 40% grape pomace in both groups (P<0.05). NDF and ADF degradation of silages were 48.43%-54.43%, 41.08%-47.42% for GAR variety, and it was in the range of 45.20-58.48%, 43.72-60.73% for SG II variety, respectively. Addition of grape pomace tended to decrease NDF degradation in sorghum-sudan grass silages, while it increased ADF degradation in GAR variety but decreased it in SG II variety (P<0.05). In studies with sorghum-sudan grass silage, it is possible to find DM degradability values of 55-65% depending on the variety and harvest time (Akdeniz *et al*., 2005; Famuyiwa and Ough, 2015). It is stated that the dry matter degradability and degradation values of different grape pomace vary between 16-39% in cattle, sheep and goats (Sarıçiçek and Ünal, 2002; Famuyiwa and Ough, 2015), it is seen that these degradability of dried grape pomace are quite low. In the present study, it is seen that the degradation values of the sorghum-sudan grass silages are similar with the literature reports. Also, the addition of grape pomace to silages in varying proportions reduces the nutrient degredation of the silages, which can be explained by the fact that the degradability of the grape pomace is lower than the nutrient degradation of the plants used in the silage.

Conclusion

As a result, it has been determined that the addition of grape pomace up to 40% to sorghum-sudan grass silages has some negative effects on silage quality, but in general, grape pomace can added to sorghum-sudan grass silages at rates of up to 20%. Thus, it can be used as an alternative roughage in ruminants.

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Author contributions

All authors contributed equally to the study.

Conflict of Interest

The author declare no conflicts of interest.

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LIVESTOCK STUDIES

RESEARCH PAPER

Welfare of Horses in Inner West Anatolia

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Abstract

This study was carried out to evaluate the feeding, management, and health practices in horse farms in Inner West Anatolia with the aspects of animal welfare. The study was carried out in 53 horse farms in Afyonkarahisar, Eskişehir, Kütahya, and Uşak provinces. The measurements, observations, and declarations of breeders were recorded on the questionnaire forms. Furthermore, a fecal parasitic examination was carried out. The breeds of horses were Arab, Thoroughbred, local, and crosses. The percentages of boxed and tied barns were 60.4 and 39.6%. The average temperature and humidity in the shelters were 20.7°C and 38.6%, the mean values for carbon dioxide, oxygen, and ammonia were measured as 848.0, 20.9, and 5.0 ppm respectively. Although antiparasitic applications were reported in the visited farms, 61.7% of the horses were found to have parasites in the parasitic examination. 90.6% of the interviewed personnel claimed that they were experienced in horse breeding but only 3.8% had knowledge about animal health and welfare. In conclusion, horse breeding in Inner West Anatolia is insufficient in terms of windows and chimney openings in shelters, shelter enrichment, paddock areas and exercise, dental care, antiparasitic applications, and trained personnel for animal health and welfare; while box dimensions, feeding standards, grooming, hoof care, tail docking, foal care and transport applications were in accordance with ideal standards.

Introduction

As interest for equestrian sports increases day by day, flat racing, javelin and pacing maintains their importance today. In addition, show jumping, dressage, endurance and eventing (three-day event) have shown improvement in recent years, as well as the number of equestrian clubs has increased and the hippotherapy centers have started to be opened. As is the case with all livestock, various situations that affect horses' well-being have manifested since their domestication. However, the number of studies on horse health and welfare is limited. When assessing horse welfare, environmental parameters such as the size of shelters and boxes, characteristics, litter, care and feeding, exercise status, and zoological parameters such as body condition scores, skin injuries, scars, and abnormal behaviors are used (Minero and Canali, 2009; Minero et al., 2015; Sommerwille et al., 2018; Czychall et al., 2019; Lesimple, 2020; Hausberger et al., 2020; Homes and Brown, 2022). When evaluating the subject of welfare in horses, it is necessary to know their behavior in natural environments. Understanding their behavior is essential in providing the most suitable environmental conditions for the animals, and optimum efficiency can be obtained from the animals with appropriate maintenance and feeding conditions (Schwean, 2005; Minero and Canali, 2009; McGreevy et al., 2018; Mellor and Burns, 2020; Auer et al., 2021; Arena et al., 2021).

In addition to European Union legal regulations, international organizations such as Food and Rural Affairs (DEFRA) (2018), Farm Animal Welfare Advisory Council (FAWAC) (2018), Royal Society for the Prevention of Cruelty to Animals (RSPCA) (2014) have determined criteria for the welfare of horses. Regulation number 28151 regarding the Welfare of Farm Animals (2011a) has been published in 2011 and regulation number 28152 regarding the Welfare and Protection of Animals During Transport (2011b) have been published in Türkiye.

This study has been carried out in order to examine the conditions of shelter, care and feeding, exercise status, personnel, herd health and some breeding practices and transportation of animals in the aspect of welfare of horse breeding enterprises in the Inner West Anatolia.

Materials and Methods

This study was approved by the Afyon Kocatepe University Animal Experiments Local Ethics Committee (49533702/323). This study was carried out at 53 horse farms in Afyonkarahisar, Eskişehir, Kütahya, and Uşak provinces. Observations and evaluations about the animal welfare were recorded on the evaluation forms prepared for this purpose.
The number of horses available in the visited equestrian establishments and the distribution of horses according to race, age and breeding purposes were determined. The sheltering standards, the dimensions of shelters, boxes, doors and windows were determined by using tape measures. The business owner was asked on their preference related to coat color and markings when raising horses.
The height of the windows was measured from the base of the shelter. Humidity, temperature, and gas concentrations in the air were measured (BW GasAlertMicro 5 IR) when animals were kept closed in the shelter. The types of shelter and shelter floor were determined, use of litter and litter material, its amount, and artificial lighting status were observed, farm owner was asked about the frequency of cleaning the shelters. It has been determined whether there is adequate shade and water in establishments that have paddock areas that enable pasturing, roaming, and socializing and whether there are individual paddocks or group paddocks according to age and sex. The owner was asked about the amount of the paddock, the annual and daily times that the horses spent on the paddock. The caretakers or establishment owners were asked about preparing the feed ration, the daily amount of roughage and concentrated feed drinking water source, and the availability of different feeding programs for pregnant horses and foals. Information about regular veterinary care, vaccination, mineral and vitamin supplements, and antiparasitic treatment applications were obtained.
Fresh feces samples were collected for parasitic examination from at least 5% of the horses raised on the farms. Feces samples were collected from the stall area of the relevant horse that was not in contact with the ground and put into separate transparent nylon bags. The collected feces were delivered to the laboratory of Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Parasitology without delay and examined macroscopically and microscopically. Fulleborn saturated brine flotation and Benedek sedimentation methods were used microscopically.
The opportunities for exercise which is very important for equine health and welfare were studied by noting whether a manege was available, the type of manege and manege floor type. The caretaker or owner was asked about the frequency of exercise of horses. Observations and evaluations about the maintenance of foals were made by asking the caretaker or owner. They were asked about the frequency of dental, grooming, and hoof care and whether the person providing dental care and the farrier were personnel of farm or independent professionals. The loading area ownership and dimensions of the transport vehicle used in horse transport within and outside the establishment, whether the loading area partitioning and side walls had sponge protection, availability of a loading and unloading ramp in the farm were determined. Feeding during journeys and transport time was also asked. Owner and personnel responsible for animal care and management, information on the total number of personnel, social security of the personnel, animal health and education, and the number of personnel trained in animal welfare was obtained.
All horses in the farms were observed for at least 20 min in order to evaluate animal-based welfare, during this observation, horses with at least one type of abnormal behavior were identified. In closed shelters where horses are tied, all horses were observed simultaneously, while horses in boxes were observed individually in each box. For the identification of horses that exhibit abnormal behavior in boxes, information provided by the animal caretaker, traces of kicking on the walls and gnawing on the troughs and fences, bites and scars on the bodies of the horses (Autoagression) were also taken into consideration.
The data obtained from the surveying and feeding standards, health and condition program, some cultivation practices, transportation, personnel and abnormal behavior were evaluated in this study. Descriptive statistics (percentages, means ± standard error of mean) were used for the analysis.

Results

The results for sheltering, feeding, health and, conditioning programs were presented in Table 1, and the other findings concerning identification, tooth, and hoof care, grooming, tail docking, foal care, transport, and personnel were showed in Table 2. Abnormal behavior rates were also given in Table 3.

Table 1

Generally, 34.0% of the farms raised horses for javelin, 30.2% for pacing, 30.2% for racing, and 5.6% for education, riding, or hippotherapy. 58.5% of the horses were Arab and Thoroughbreds and 41.5% were Turkish native horses or Arab crosses. The average herd size was 10.3 animals and the ratios of mares, stallions, geldings, and foals were 42.7 17.8, 0.7, and 38.8 respectively. At the time of selection, 14% and 15% of the farmers considered coat color (chestnut and bay) and the markings (low or no leg markings and black hoofs) on the horses as criteria.

Table 1: Findings about shelters, feeding, exercise and herd health in equine establishments

Factor	Variable	Value
Sheltering Standards	***Shelter conditions***	
	Shelter type (%)	Tied: 39.6, Box: 60.4
	Shelter width and length (m) (x̄ ± Sx̄)	(4.3±0.52) × (7.5±2.08)
	Box width and length (m) (x̄ ± Sx̄)	(3.6±0.11) × (3.7±0.11)
	Window width and length (m) (x̄ ± Sx̄)	(0.6±0.03) × (0.8±0.05)
	Height of window from floor (m) (x̄ ± Sx̄)	1.7±0.07
	Door height and width (m) (x̄ ± Sx̄)	(1.4±0.12) × (2.0±0.04)
	Artificial lighting (%)	Yes: 100.0, No: 0.0
	Visual contact among horses (%)	Yes: 37.7, No: 62.3
	A specific box/stall per horse (%)	Yes: 90.6, No: 9.4
	Box equipped with toy (%)	Yes: 0.0, No: 100.0
	Quarantine section (%)	Yes: 20.8, No: 79.2
	Shelter internal air status	
	Shelter internal temperature (ºC) (x̄ ± Sx̄)	20.7±0.38
	Shelter internal humidity (%) (x̄ ± Sx̄)	38.6±0.61
	Shelter internal carbon dioxide (ppm) (x̄ ± Sx̄)	848.0±78.41
	Shelter internal oxygen (ppm) (x̄ ± Sx̄)	20.9±0.00
	Shelter internal ammonium (ppm) (x̄ ± Sx̄)	5.0±0.75
	Shelter floor type (%)	Concrete: 47.2, Soil: 45.2, Rubber: 5.7,Wood: 1.9
	Litter	
	Litter usage (%)	Yes: 62.4, No: 37.6
	Litter material (%)	Straw: 51.5, Sawdust: 24.2, Sheep manure: 9.1, Straw+ Sheep manure:6.1, Sawdust + Sheep manure: 9.1
	Litter amount (kg) (x̄ ± Sx̄)	10.6±1.80
	Litter changing frequency (%)	Daily: 51.5, One time/two days: 9.1, One time/week: 21.2, One time/month: 18.2
	Outing area	
	Open outing area (%)	Yes: 66.0 , No: 34.0
	Average outing area (m^2) (x̄ ± Sx̄)	24193.0±11131.13
	Paddock on outing area (%)	Yes: 62.9, No: 37.1
	Outing area surface (%)	Soil: 100.0
	Outing area vegetation cover (%)	Yes: 11.4, No: 88.6
	Outing area trees (%)	Yes: 8.6, No: 91.4
	Water supply on outing area (%)	Yes: 65.7, No: 34.3
	Departure and return times from outing area	Departure: 08:00-08:30, Return: 17:00-17:30
	Departure occasions to the outing area	All days weather permitting
Feeding Standards	Ration preparation (%)	Traditional mixed feed in the establishment: 86.8, Using a program in the establishment: 7.5, Veterinary surgeon: 5.7
	Daily roughage amount (kg/head) (x̄ ± Sx̄)	3.3±0.24
	Daily concentrated feed amount (kg/head) (x̄ ± Sx̄)	4.7±0.22
	Drinking water source (%)	City water: 34.0, Well water: 64.1, Flowing water: 1.9
	Feeding every day at the same time (%)	Yes: 98.1, No: 1.9
	Feeding every day at the same sequence (%)	Yes: 98.1, No: 1.9
	Separate feeding for gestating mares and foals (%)	Yes: 34.0, No: 1.9, No pregnant animals: 64.1
Health and Condition Program	***Condition***	
	Veterinary care (%)	Yes: 86.8, No: 13.2
	Vaccination (%)	Yes: 77.4, No: 22.6
	Vitamin and mineral supplements (%)	Yes: 83.0, No: 17.0
	Fly fighting method (%)	Spray: 77.3, Electrical device: 1.9, Sticky tape: 1.9, No: 18.9
	Manege (%)	Yes: 9.4, No: 90.6
	Manege type (%)	Closed: 40.0, Open: 60.0
	Manege floor (%)	Soil: 40.0, Sand: 60.0
	Exercise frequency (%)	Two times/day: 20.0,One time/day: 60.0,One time/week: 20.0
	Anti-parasite action	
	Anti-parasite treatment program (%)	Yes: 64.2, No: 35.8
	Anti-parasite treatment method (%)	Paste: 47.1, Pill: 23.5, Paste+pill: 11.8, Paste+injection: 17.6
	Rate of parasites observed in fecal samples	
	Flotation (%)	Yes: 61.7, No: 38.3
	Sedimentation (%)	Yes: 5.4, No: 94.6
	Maximum rate of parasites observed in fecal samples	
	With flotation (%)	*Strongylidae sp.*: 32.2
	With sedimentation (%)	*Eimeria sp.* : 2.7

Table 2 and Table 3

The proportions of farms with tied or boxed housing were 39.6%, and 60.4%. The percent of farms where horses facing one another with visual contact were 38.7%. No farm had toys like balls, tires, or any other objects. The ground was only soil in 82.8% of the roaming areas. It was seen that clover, vetch, and straw

Table 2: Findings related to identification, tooth, hoof care, grooming, tail docking, foal care, transport and personnel in horse breeding establishments

Factor	Variable	Value
Identification	Identification (%)	Yes: 30.2, No: 69.8
	Identification method (%)	Microchip: 100.0
Dental, hoof care, grooming and tail docking	Dental care (%)	Yes: 24.5, No: 75.5
	Dental care done by (%)	Veterinary surgeon: 15.4, Owner: 84.6
	Farrier (%)	Own farrier: 13.2, Outsourced: 81.1, Owner: 5.7
	Hoof trimming (%)	One time/one-two months: 60.3, One time/three-four months: 34.0, One time/six months: 5.7
	Hoof disease (%)	Yes: 0.0, No: 100.0
	Posture Disorder (%)	Yes: 20.8, No: 79.2
	Gait defect (%)	Yes: 0.0, No: 100.0
	Grooming (%)	Two times/day: 1.9, One time/day: 41.5, One time/two days: 11.3, One time/week: 30.2, One time/month: 3.8, When dirty: 11.3
	Grooming person (%)	Stableman: 30.2, Owner: 69.8
	Time of grooming (%)	Every day same time: 62.3, Irregular: 37.7
	Grooming session time (min) ($\bar{x} \pm S\bar{x}$)	18.5±1.10
	Tail docking (%)	Yes. 0.0, No: 100.0
Foal care	Foals kept with mothers (%)	Yes: 100.0, No:0.0
	Weaning age (month) ($\bar{x} \pm S\bar{x}$)	6.4±0.25
	Weaning method (%)	Abruptly: 95.5, Gradually: 4.5
Transport	Transport vehicle (%)	Yes: 28.3, No: 71.7
	Width and length of vehicle interior loading area (m) ($\bar{x} \pm S\bar{x}$)	(1.9±0.14) × (4.6±0.75)
	Width and length of vehicle interior partition (m) ($\bar{x} \pm S\bar{x}$)	(0.8±0.00) × (2.2±0.00)
	Shortest transport distance (km) ($\bar{x} \pm S\bar{x}$)	146.8±52.08
	Longest transport distance (km) ($\bar{x} \pm S\bar{x}$)	395.1±61.32
	Feed and water supply in vehicle (%)	Yes: 20.0, No: 80.0
	Loading and unloading ramp (%)	Yes: 100.0, No: 0.0
	Internal vehicle foam coating (%)	Yes: 100.0, No: 0.0
Personnel	Total number of personnel (%)	One-two people: 77.4, Three-four people: 13.2, Five-ten people: 9.4
	Personnel's social security (%)	Yes: 98.1, No: 1.9
	Personnel with instruction regarding animal health (%)	Yes: 3.8, No: 96.2
	Personnel with instruction regarding animal welfare (%)	Yes: 3.8, No: 96.2

Table 3: Findings regarding the rate of establishments where abnormal horse behavior is observed

Factor	Variable	Value (%)
Abnormal Behavior	Rate of establishments where abnormal behavior is observed	39.6
	Distribution of the types of abnormal behavior observed in establishments	
	Cribbing	20.8
	Wood chewing	18.9
	Kicking against the stall walls	17.0
	Weaving	17.0
	Manure eating and soil eating	13.2
	Self-mutilation (Autoaggression)	13.2
	Headshaking	9.4
	Tongue playing	9.4
	Heightened aggressiveness	9.4
	Excessive pawing	7.5
	Stall walking, fence walking and figure eight walking	7.5
	Excessive eating (Polyphagia nervosa)	5.7
	Excessive tail swishing	1.9
	Distribution of establishments according to the type of Observed abnormal behavior	
	1 abnormal behavior	11.3
	2 abnormal behaviors	13.2
	3 abnormal behaviors	3.8
	6 and more abnormal behaviors	11.3

were used as roughage in rations, while barley, bran, oat, and pelleted feeds were fed as concentrate. The most common postural deformities were perpendicular hoof and extroverted stance. Only 9.4% of the people performing shoeing procedures were trained in farriery, horse health, and welfare. In all farms, foals were kept with their mothers and the weaning age of foals showed variation (5-6 months in 68.2% and 7-8 months in 27.3%). The inner surfaces of the transport vehicles belonging to the enterprises were covered with foam and had a loading ramp. While 100% of the interviewed hostler reported that they had experience in livestock breeding and 90.6% in horse breeding, only 3.8% of them reported that they had knowledge about animal health and welfare.

Discussion

The average horse box dimensions were 3.6 x 3.7 m and were compliant with the international standards advised (3.65 x 3.65 m) for warm-blooded horses (NEWC, 2009; DEFRA, 2018; FAWAC, 2018). Similarly, indoor temperature and humidity values (20.7°C and 38.6%) were between the optimum values (5-30°C and 30-70%) reported for horses (Morgan et al., 1997; Ödber and Bouissou, 1999; Schwean, 2005; Parker, 2007; NFACC, 2018; Miller et al., 2019). The average ammonia level measured in barns was 5.0 ppm and lower than the recommended maximum of 10 ppm (NFACC, 2018; New Zealand Government, 2018; Miller et al., 2019) for horse barns. It was determined that the ventilation openings such as windows and chimneys were insufficient in the shelters, however, the reason for the good indoor air quality during the visit was the number of horses housed lower than the capacity of the barn (Curtis et al., 1996; Schwean, 2005). Good litter and manure removal frequency were also had a positive impact (Schwean, 2005; Parker, 2007).

While the litter was not available in one-third of the farms, 24.3% of them used dry sheep manure or a local litter obtained by mixing straw and sawdust. Farmers reported that they preferred this natural litter because the feces was dried during summer under the sun and composted as well as its ready availability and low cost. There was a wide variation in the use of litter among the farms and poor litter management has been reported to reduce shelter comfort and horse welfare.

24193.0 m2 per farm and 1660.3 m2 per horse were deemed to be inadequate. These values were lower than the 5000-10000 m2 / head values indicated as optimum outing and grazing areas reported for horses(DEFRA, 2018; NFACC, 2018). Furthermore, nutritional vegetation, such as grass or meadows, is also rare in these outing areas (11.4%). These results indicate that horses on farms cannot carry out feed exploration and collective group grazing activities to a significant degree. Although the existence of an outing area allows horses to exhibit some important comfort behaviors such as rolling around in the soil, 62.9% of the farms with an outing area keep horses in individual paddocks and therefore horses are prevented from exhibiting social behavior that is very important (Arnold and Grassia, 1982; Carson and Wood-Gush, 1983; Boyd, 1991; Araba and Crowell-Davis, 1994). Moreover, most of the pacing and javelin horse breeders had only one horse. The results show that the farms feed the horses sufficiently (Henderson and Waran, 2001). However, horses were not able to ad libitum access to fresh and clean water and may even be dehydrated for long hours. In the majority of the farms (77% or more), owners reported that they provided regular veterinary care to horses, made compulsory vaccinations and applied routine vitamin and mineral supplements. Contrary to these declarations, 61.7% of the horses determined at the appropriate sampling rate were found to have parasites in their feces. These results suggest that the health protection and antiparasitic methods used in the farm are not enough for successful breeding. Parasitic infections adversely affect horse health and welfare as in all livestock. The lack of a quarantine section in 79.2% of the farms constitutes a significant risk (DEFRA, 2018; NFACC, 2018; New Zealand Government, 2018). However, 81.1% of the farms reported that they used different methods to fight flies (Miller et al., 2019; Parker, 2007).

It was determined that there are few farms having a manege and horses exercise regularly (only 9.4%). In particular, due to inadequate exercise and limited social contact, an increase in abnormal behavior was observed in horses (Krzak et al., 1991; Jorgensen and Boe, 2007; DEFRA, 2018; Lesimplea et al., 2020). As a matter of fact, the abnormal behavior rate of farms was 39.6%. The variety and number of abnormal behaviors in horses is an important parameter indicating low welfare (RSPCA, 2014; Arena et al., 2021; Seabra et al., 2021; Krueger et al., 2021; Brolin, 2022). Only one or a few horses were determined in majority of farms throughout the study. This phenomenon may be thought to result in boredom and loneliness and therefore abnormal attitudes in the animals. Visual contact or environmental enrichment can reduce boredom (Mills, 2002; McAfee et al., 2002; Mills and 2005; Eicher, 2022; Mejia et al., 2022). Abnormal behaviors pose risks for both horses as well as caretakers. Buckley et al. (2010) reported that 60% of rider injuries were caused by behavior problems and the performance of horses with behavior problems decreased. Ödber and Bouissou (1999) reported that behavior problems had a significant role in the number of horses sent to the slaughterhouse in Italy.

In 30.2% of the farms, horses were identified with microchips. These farms are engaged in raising horses for flat racing and according to the Regulation regarding the Registration of Arab and Thoroughbred horses into the Studbook, their Export, and Import, Arab and Thoroughbred foals born in Türkiye in2006 and later must be identified with microchips in order to be included in the studbook (Türkiye Tarım ve Orman Bakanlığı, 2021).

The hoof trimming frequency indicated that shoeing and hoof care are managed well (Parker, 2007; DEFRA, 2018; FAWAC, 2018; NFACC, 2018; Miller et al., 2019). Frequent grooming in the farms has highly positive effects for horse welfare. Grooming contributes to between interactions of human and animal when disturbing substances such as dirt, mud, soil, fertilizer, and straw that accumulate on the bodies of horses are being removed and the grooming is considered as a need of horse (Miller et al., 2019). It is thought that the lack of dental care in a large majority of farms may affect animal welfare and performance negatively as it will cause tooth discomfort in horses and weight loss by preventing chewing. The absence of tail docking was also positive for horse welfare (The United Kingdom Legislation, 1949; Norwegian Animal Welfare Act, 2011; Finnish Centre for Animal Welfare, 2013; Austria Federal Act on the Protection of Animals, 2014). It is necessary for the horse to protect itself from flies. Tail is used to display some attitudes such as aggression, social behavior, and joy in communication with other horses and humans (Christie et al., 2004; Levebvre et al., 2007; Türkiye Tarım ve Orman Bakanlığı, 2011a).

Foals are weaned at the age of 6 months earliest. Keeping foals with their mothers until weaning, prevents stress in mares and foals (NFACC, 2018). However, the application of abrupt weaning in the farms is a practice that is stressful to foals and reduces their welfare (Schwean, 2005; Parker, 2007; New Zealand Government, 2018). Only one-third of the enterprises had their own transport vehicle that is foam-coated and had loading and unloading ramps to prevent the horses from being injured as a result of slipping, falling, and impact. The trailer area (1.76 m2) is in compliance with EU requirements (1/2005/EC) and Regulation on Welfare and Protection of Animals During Transport (Türkiye Tarım ve Orman Bakanlığı, 2011b) in Türkiye.

All of the interviewed personnel being experienced regarding farm animals. Most of them (90.6%) being experienced in horse breeding stated that an advantage in terms of providing good horse care. However, the fact that only 3.8% of the farms had technical personnel trained in animal health and welfare may be considered a disadvantage.

Conclusion

It is concluded that sheltering standards in the visited horse farms were good with the exception of window and chimneys. The feeding practice is thought to be adequate. Although the living area in individual boxes for the animals was sufficient, no enrichment was observed. Abnormal behaviors resulting from inadequate housing is an indication of high social stress in horses. It has been concluded that the absence of tail docking, frequent grooming, hoof care and good transport conditions reflect high standards of welfare in farms. In contrast, the lack of dental care, high parasite burden and potentially poor human-animal interactions caused by caretakers not trained in animal health and welfare pointed to low welfare standards.

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Conflict of Interest

The authors declared that there is no conflict of interest.

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