T.R.
NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF ANIMAL PRODUCTION AND TECHNOLOGIES

EFFECT OF CABBAGE AND KOHLRABI LEAF SILAGES ON IN VITRO AND CLASSICAL NUTRIENT DIGESTIBILITY IN AKKARAMAN RAMS AS AN ALTERNATIVE FEED SOURCE

MUHAMMAD ZEESHAN AKRAM

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MASTER THESIS

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Supervisor

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Muhammad Zeeshan Akram tarafından **Assist. Prof. Dr. Sema YAMAN FIRINCIOĞLU** danışmanlığında hazırlanan **“Alternatif yem kaynağı olarak lahana ve alabaş yaprağı silajlarınınakkaraman koçlarında in vitro ve klasik besin madde sindirilebilirliğine etkisi”** adlı bu çalışma jürimiz tarafından Niğde Ömer Halisdemir Üniversitesi Fen Bilimleri Enstitüsü Hayvansal Üretim ve Teknolojileri Ana Bilim Dalı’nda Yüksek Lisans tezi olarak kabul edilmiştir.

(The study titled **“Effect of cabbage and kohlrabi leaf silages on in vitro and classical nutrient digestibılity in akkaraman rams as an alternative feed source”** and presented by **Muhammad Zeeshan Akram** with the help of supervisor **Prof Assist. Prof. Dr. Sema YAMAN FIRINCIOĞLU,** has been found as Master thesis by the jury at the Department of Animal Production and Technologies of Niğde Ömer Halisdemir University Graduate School of Natural and Applied Sciences.)

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Prof. Dr. Murat BARUT

DIRECTOR

THESIS CERTIFICATION

I proclaim that the thesis entitled *“Effect of cabbage and kohlrabi leaf silages on in vitro and classical nutrient digestibility in Akkaraman rams as an alternative feed source”* has been written by me and that to the best of my knowledge and belief. All the information presented in this thesis is scientific and corresponding to academic rules. The results embodied in this thesis have not been submitted to any other university or institute for the award of any degree or diploma. All the assistances and sources that helped me have been acknowledged in the thesis.

Muhammad Zeeshan AKRAM

ÖZET

ALTERNATİF YEM KAYNAĞI OLARAK LAHANA VE ALABAŞ YAPRAĞI SİLAJLARININAKKARAMAN KOÇLARINDA İN VİTRO VE KLASİK BESİN MADDE SİNDİRİLEBİLİRLİĞİNE ETKİSİ

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Çalışma, lahana ve alabaş yaprakları ve silajlarının kimyasal bileşimini, in vivo sindirilebilirliğini, in vitro sindirilebilirliğini, toplam fenolik içeriğini ve antioksidan aktivitesini kontrol etmek için yapıldı. İn vivo sindirilebilirlik deneyleri için R1 (Alfalfa 100), R2 (Yonca ve lahana silajı 50:50) ve R3 (Yonca ve lahana + alabaş silajı 50:50) üç rasyon kullanılmıştır. Taze lahana ve alabaş yaprakları ve silajları düşük DM, NDF ve ADF'ye ancak yüksek CP içeriğine sahipti. Silajların fiziksel özellikleri iyidir ve pH değerleri 5,4 ile 5,7 arasında değişmiştir. DM, OM ve CP'nin in vivo sindirilebilirlik değerleri, R2 ve R3'te R1'e göre benzer ve anlamlı olarak daha yüksek ($P < 0.05$) iken, NDF ve ADF sindirilebilirlik değerleri R2'de diğer rasyonlara göre anlamlı olarak daha yüksekti ($P < 0.05$). Her iki silajın da in vivo besin sindirilebilirlik değerleri, yoncaya göre daha yüksekti. In vitro gerçek sindirilebilirlik, taze lahana ve alabaş yapraklarının ve silajların OM ve NDF sindirilebilirliği sırasıyla% 90,% 65 ve% 62'nin üzerindeydi. Taze alabaş yapraklarının toplam fenolik içeriği ve antioksidan aktivitesi diğerlerine göre önemli ölçüde daha yüksek ($P < 0.05$) idi. Lahana ve alabaş yaprakları ve bunların silajları, geviş getiren hayvanlar için geleneksel olmayan mükemmel yem kaynaklarıdır.

Anahtar Sözcükler: lahana, alabaş, silaj, sindirilebilirlik, koyun

SUMMARY

EFFECT OF CABBAGE AND KOHLRABI LEAF SILAGES ON IN VITRO AND CLASSICAL NUTRIENT DIGESTIBILITY IN AKKARAMAN RAMS AS AN ALTERNATIVE FEED SOURCE

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This study was conducted to examine the chemical composition, *in vivo* apparent nutrient digestibility, *in vitro* digestibility, total phenolic content, and antioxidant activity of cabbage and kohlrabi leaves and their silages. Three rations R1 (Alfalfa hay 100), R2 (Alfalfa hay and cabbage leaves silage 50:50), and R3 (Alfalfa hay and cabbage + kohlrabi leaves silage 50:50) were used for *in vivo* digestibility trials. Fresh cabbage and kohlrabi leaves and their silages had low DM, NDF and ADF but high CP content. Both silages had good physical characteristics and pH ranged from 5.4 to 5.7. *In vivo* digestibility values of DM, OM and CP were similar and significantly higher ($P<0.05$) in R2 and R3 as compared to R1 while NDF and ADF digestibility values were significantly higher ($P<0.05$) in R2 than other rations. *In vivo* nutrient digestibility values of both silages were observed higher than alfalfa hay. *In vitro* true digestibility, OM and NDF digestibility of fresh cabbage and kohlrabi leaves and their silages had greater than 90%, 65%, and 62%, respectively. Fresh kohlrabi leaves had significantly higher ($P<0.05$) total phenolic content and antioxidant activity than others. It is concluded that cabbage and kohlrabi leaves and their silages may offer excellent potential as non-conventional feed source for ruminants.

Keywords: cabbage, kohlrabi, silage, digestibility, sheep

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SYMBOLS AND ABBREVIATIONS

Symbols	**Abbreviations**
%	Percentage
℃	Degrees celsius
g	Gram
Kg	Kilogram
L	Liter
mg	Milligram
mL	Milliliter

Abbreviations	**Descriptions**
SEM	Standard error mean
TDN	Total digestible nutrient
CP	Crude protein
DM	Dry matter
CF	Crude fiber
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
ADL	Acid detergent lignin
TVFA	Total volatile fatty acids
TPC	Total phenolic contents
MH	Million hectares
AU	Animal units
MT	Million tons
ANF	Anti-nutritional factors
GE	Gross energy
CS	Cabbage leaves silage
CKS	Cabbage + kohlrabi leaves silage
IVTD	*In vitro* true digestibility
TEAC	Trolox equivalent antioxidant capacity
ABTS	2,2'-azinobis 3-ethyl-bezothiazoline 6 sulfonate

CHAPTER I

INTRODUCTION

Livestock production is an important sector of agriculture throughout the world. It contributes substantially to the national gross domestic product (GDP) of countries (Rehman et al., 2017). It acts as a backbone of the rural economy in underdeveloped countries which needs improvement using advanced technologies and balanced nutrition (Alabi et al., 2019; Rothschild and Plastow, 2016). Animal nutrition has a pronounced impact on animal production as well as on human health and the environment. The feed is recognized as the most important component of livestock production systems contributing up to 70% of the cost of production. The efficient utilization of available feed resources is very important as it is a primary determinant of animal performance and productivity (Mahesh and Mohini, 2014). Moreover, the availability and utilization of animal feed possess multifaceted implications in terms of farm economics, environmental sustainability, animal health, welfare, product quality, and safety (Distel and Villalba, 2018).

Poverty, food deprivation and climate change are prevalent issues of this planet. The livestock sector is a major contributor in raising the rural economy and supports the livelihoods of people in different ways providing a range of many important benefits (Rehman et al., 2017). The demand for animal products such as meat, milk and eggs is increasing globally to a greater extent and will continue to increase in the near future (McClements, 2020; Enahoro et al., 2018; Mahesh and Mohini, 2014). Furthermore, the human world population will be more than 10 billion and food demand will increase anywhere between 59 to 98% by 2050 (Devendra and Leng, 2011). Correspondingly, it encourages the livestock population by 70%. To accomplish the targeted level of production, efficient livestock feeding is very important as feed is a major determinant of livestock production (Mahesh and Mohini, 2014; Pelletier and Tyedmers, 2010). Whereas, livestock production is restrained globally due to inadequate supply of feed for optimum production (Röös et al., 2017). Moreover, land used for fodder production is not expected to increase in the near future (Qi et al., 2017). Rapid depletion of farmable land due to urbanization is leading towards reduction in the supply of animal feed resulting in low animal productivity (Schiere, 2010; Saritha and Arora, 2012). The intensive livestock production system in developed countries is focused on feeding grains and soya which could otherwise be directly consumed by human beings (Mottet et al., 2017). Moreover, the expenses of conventional feed ingredients such as green fodders and grains are continuously increasing globally. All these circumstances encourage the farmers to feed livestock on human inedible feed materials that do not compete with human nutrition. Among those feedstuffs, crop residues, pastures, and agro-industrial byproducts are major contributors that can reduce further deforestation and land-use change.

The use of local feed resources, crop waste, and residues as livestock feed is a necessary precondition for commercial production. Crop residues are a valuable source of animal feed and their utilization by grazing is very effective in returning plant nutrients to the soil. Crop residues produced in a significant quantity that can potentially be used as alternatives of conventional feedstuff for ruminant feeding and generate economic benefits for the farmers by selling crops in the market and using crop residues as animal feed (Akram and Firincioğlu, 2019; Ali et al., 2019). Moreover, farmers produce a large quantity of crop residues, which possess a good nutrient profile, could be used as alternatives to conventional feedstuffs during feed shortage periods (Akram and Firincioğlu, 2019). Generally, farmers either burn the crop waste and residues in the field or plow them in the soil. The environment is being compromised due to the burning issues of excessive crop waste in recent years. This is a major cause of climate change in and producing problems like smog in winter seasons, which leads to respiratory problems (Singh et al., 2020). Thus, the production of vegetables and fruits is constantly growing, making byproducts and waste abundantly available. These resources could be used as potential feed for ruminant diet. This strategy would generate the financial benefits for farmers and contribute to alleviating the environmental issues associated with their elimination (Devi et al., 2017). Furthermore, an insufficient supply of fodder can be a second reason behind the utilization of crop residues. In some countries, these valuable by-products could be used as potential ruminant feed during lean periods to meet the demand of animals (Akram and Firincioğlu, 2019). The utilization of by-products is however limited due to the poor understanding of their nutritional and economic values as well as their proper use in ruminant rations (Simanihuruk et al., 2019). In Niğde, approximately 109000 tons of cabbage are produced annually. Out of this only 11% is being used as animal feed (Personal communication).

Discarded cabbage (*Brassica oleracea var. capitata*) and kohlrabi (*Brassica oleracea var. gongylodes*) are vegetable by-products available in the markets and agriculture fields during harvesting. Preliminary researches have reported that brassica plants like cabbage leaves have high crude protein (CP) but low neutral detergent fibers (NDF) and lignin contents, which increase their dry matter (DM) intake (Katongole et al., 2011). The chemical analysis of brassica vegetables particularly cabbage, cauliflower, and kale revealed that these plants have a range of 7.0-14.0% of DM. Organic matter (OM), ash, ether extracts (EE), total digestible nutrients (TDN), and CP could be present with the range of 82.5-88%, 7.04-20.47%, 1.89-2.66%, 74-84%, and 10.36-23.6%, respectively. However, 11.12-24.6% crude fiber (CF), 20-34% NDF, 15.3-23.5%ADF, 4.22% (ADL), 5.5-11% hemicellulose, 12.5-16% cellulose have reported in former studies (Brito et al., 2020; Mahgoub et al., 2018; Bakshi et al., 2007; Wadhwa et al., 2006). Using brassica vegetable wastes in the form of either fresh or processed (hay or silage) may act as good unconventional feedstuffs for ruminants and decrease the feed cost, equivalent to any conventional feed like clover hay. El-Shinnawy et al. (2011) revealed that cabbage silage and hay improved with urea had significant-good nutrient digestibility in rams and improved significantly TDN, CP, and nitrogen balance values. Moreover, total volatile fatty acids (TVFA) concentration was found high with low ammonia nitrogen (NH3-N) value in silage and hay. Moreover, past studies reported 65-70% of the in-vivo digestibility of cabbage in ruminants (El-Shinnawy et al., 2011). However, cabbage waste and leaves are extensively fermented in the rumen in-vitro (Eván et al., 2019). Mekasha et al. (2002) observed 80.4% *in vitro* DM digestibility of cabbage. While, Eván et al. (2019) reported above 91.9% rumen degradability of DM for cabbage at 12 hours of in situ incubation. Whereas, digestibility of DM in the intestine was 45.7%. In terms of protein degradability, it ranged from 61.4 to 90.2%.

Brassica plants are commonly used as food and among the top 10 economically important crops in the world. They have been added to a healthy diet as an important component due to their high level of nutrients and health beneficial phytochemicals such as phenolics, glucosinolates, and vitamins. Many experimental analyses have revealed that intake of brassica vegetables is effectively linked with the reduction of degenerative diseases. The antioxidant properties of phenolic contents of these plants play a vital role in prevention of several degenerative diseases linked with oxidative stress like cardiovascular, cancer, and immune dysfunction (Ravindran et al., 2019). The easiest method to determine the total phenolic content (TPC) is the Folin-Ciocalteu method. Karoui et al. (2018) reported 205.7 mg GAE)/100 g-^{1} DW TPC in white cabbage. More as, Liang et al. (2019) reported TPC in different varieties of cabbage ranged from 86.64 to 153.94 mg GAE/100 g-^{1} DW.

Brassica vegetables contain multiple secondary compounds like S-methyl-L-cysteine sulphoxide and glucosinolates (Neugart et al., 2018). These compounds are toxic to animals to some extent and can reduce their productivity and feed intake (FI) when fed in high quantities (Taljaard, 1993). However, it is reported that rumen microorganisms can detoxify these secondary metabolites if better concentration level and gradual feeding of cabbage is offered to animals (Eván et al., 2019). To maximize the nutritional value of cabbage residues, the overall processing steps must be well defined to obtain the desired physical and nutritional quality. The waste and leaves of cabbage contain high moisture content, which limits its use for utilization in animal feeding. Moreover, leaves are more prone to spoil and costly to transport and handle due to high moisture level. However, they can be sun-dried before getting introduced in ruminant diets or ensiled (Nkosi et al., 2016; Bakshi et al, 2016).

The unavailability of good quality fodder is the main problem in improving livestock production under-resourced poor areas around the world. Farmers are offering whatever feedstuffs are available to their animals because of unaffordable feed costs of conventional feedstuffs. It is therefore dire need of time to find alternatives with affordable cost which can be fed to animals for increasing productivity (Akram and Firincioğlu, 2019). The utilization of crop residues as roughages has been the subject of intense research since the 1970s. Despite this, it appears little evidence that large research has resulted in great utilization of crop residues in developing countries. Among agriculture field crop residues, leaves of cabbage and kohlrabi have good nutrient profiles and are common in various regions of Turkey. The main objective of this study was to check the possibility of utilizing cabbage and kohlrabi leaves as a non-conventional feed source for ruminants and trying to improve their utilization through the ensiling process. Moreover, the parameters studied were nutritional composition, silage quality, *in vivo* apparent nutrient digestibility coefficients, *in vitro* true digestibility, *in vitro* OM digestibility, *in vitro* NDF digestibility, total phenolic content, and antioxidant activity of cabbage and kohlrabi leaves and their silages.

CHAPTER II

LITERATURE REVIEW

2.1 What are Crop Residues?

Crop residues are byproducts or waste materials of crops, vegetables, and fruits left on agricultural land after the crop has been harvested. There are two types of crop residues including cultivated land wastes and agro-industrial process residues. Agricultural crop residues include leaves, stalks, stubble, stems, and seed pods. Parts of plants and crops grown for food, fiber, and animal feed do not produce most of the phytomass annually how much crop residues do. Globally, more than half of the DM is produced in the form of cereal and legume straws, tops, stalks, leaves, and shoots of tubers (Akram and Firincioğlu, 2019).

2.2 Crop Residues as Alternative to Conventional Feedstuffs

Food shortages and famine are becoming endemic in developing countries. It is associated with urbanization, industrialization, and reduction in farmable land. The demand for animal products such as milk, meat, and eggs is increasing globally to a greater extent with the increase in the human population (Mahesh and Mohini, 2014). To accomplish the targeted level of production, efficient livestock feeding is very important as feed is a major determinant of livestock production and accounts for almost 65-70% of recurring expenditures (Mahesh and Mohini, 2014; Pelletier and Tyedmers, 2010). Whereas, livestock production is restrained globally due to the inadequate supply of feed for optimum production. Land used for fodder production is not expected to increase in the near future. Moreover, the expenses of conventional feed ingredients such as green fodders and grains are continuously increasing globally. From that perspective, crop residues have great potential as ruminant feed sources.

2.3 Brassica Family Origin and Characteristics

Brassica (B.) is a genus in the Brassicaceae family commonly known as cruciferous vegetables. This genus is either annual or biennial and important as both agricultural and horticultural crops including several weeds. Most of the brassica varieties are usually used for food like cabbage, cauliflower, Brussels sprouts, kohlrabi, broccoli, kale, rape, rutabaga, turnip, and choy sum. Some varieties contain seeds that are used for vegetable oil production include canola oil and mustard oil. Brassica plants particularly B. carinata, B.juncea, B. oleracea, B. napus, B. nigra, and B. rapa have been the subject of much scientific interest for their agricultural and economic importance. This genus is widely spread around the world but native to Western Europe and the temperate region of Asia but commonly found in Mediterranean regions (Šamec and Salopek-Sondi, 2019; Khurshid et al., 2019; Šamec et al., 2017).

2.4 Nutrient Profile of Brassica Vegetables

The chemical analysis of brassica vegetables particularly cabbage, cauliflower, and kale revealed that these plants have a range of 7.0-16.0 DM percentage. OM, ash, EE, TDN, and CP could be present with the range of 82.5-88%, 7.04-20.47%, 1.89-2.66%, 74%, and 10.36-23.6%, respectively. In the case of fibrous content, CF (11.12-24.6%), NDF (20-34%), ADF (15.8-23.5%), ADL (4.22%), hemicellulose (5.5-11%), and cellulose (12.5-16%) have been reported in various studies (Mahgoub et al., 2018; Bakshi et al., 2007; Wadhwa et al., 2006).

2.5 Status of Crop Residues in Turkey

Turkey is located between Europe and Asia having an area of 78.35 million hectares (MH) out of which 76.96 MH is a land zone. The total agricultural land is decreasing gradually for the last two decades. Total utilized agricultural land is 37.80 MH of which 18.93 MH for cereals and other crop products, 0.784 MH for vegetable gardens, 0.005 MH for ornamental plants, 3.462 MH for fruits, beverages, and spice crops, and 14.62 MH land under permanent meadows and pastures (TUIK, 2018). The share of animal husbandry in Turkey’s agriculture sector is about 30%. During grazing seasons, most of the animals depend on harvest residues and rangelands for feeding. Rangelands are very important particularly during crop growing seasons due to the unavailability of meadows or alternative feed resources for livestock. Turkey’s ruminant population consists of 17,042,506 cattle, 46,117,399 small ruminants (TUIK, 2018). No significant change has observed in cattle but small ruminants number reduced from 1985 to 2010. After 2010, the small ruminant number is gaining a boost and increasing gradually. Due to advanced agricultural techniques and mechanization, equids number decreased in the country. Turkey has more than 10 million animal units and round a year roughage demand is about 37 million tons (Holechek et al., 2004). The average altitude of Turkey is about 1000 meters and the grazing season for animals is merely 180 days (Altin et al., 2011). Out of 10 million animal units (AU), around 7.5 million AU are getting their feed from rangelands and approximately their requirement is 13.5 million tons (MT) of roughages. The contribution of rangelands in Turkey is about 7.6 MT of roughages but that amount is far away to cover the demands of animals (Koc et al., 2012). There is a huge gap in the supply and demand of roughages during the grazing season in Turkey. This demand is accomplished with the help of low quality crop stubble, fallow land, and understory plantation. Roughly, 2.65 million AU ruminants especially cattle are reared in the intensive system and their roughages demand during the summer season reached 4.75 MT. On the other side, in winter, 18.75 MT of roughages are required and the total roughage need for an intensive rearing system is about to 25.5 MT. The total production from hay lands (meadow plus forage crop cultivation) is about 13.3 MT in the country. Accumulatively, there is a 12 MT roughage gap in Turkey in summer and winter. Sugar beet leaves, vegetable residues and understory plantations are some of the substitutes for roughage sources which makes up an amount of 5.0 MT. Eventually, roughage gap of 7.2 MT is covered by cereal straw. (Koc et al., 2012).

2.6 Total Phenolic Contents in Brassica Vegetables

Brassica plants are among the top 10 economically important crops all over the world. They have health-promoting bioactive components such as phenolics, glucosinolates, and vitamins. These properties make them add to the human diet as an important component. Many experimental analyses have revealed that intake of brassica vegetables is effectively linked with the reduction of degenerative diseases. The antioxidant properties of phenolic contents of these plants play a vital role in preventing several degenerative (Ravindran et al., 2019). The easiest method to determine the TPC is the Folin-Ciocalteu method. Karoui et al., (2018) reported 205.7 mg GAE)/100 g-^{1} DW TPC in white cabbage. More as, Liang et al., (2019) reported TPC in different varieties of cabbage ranged from 86.64 to 153.94 mg GAE/100 g-^{1} DW. Similarly, Isabelle et al. (2010) observed 186 mg GAE/100 g-^{1} DW TPC in red cabbage. TPC of fresh kohlrabi leaves was found ranged from 164.7 to 275.8 mg GAE/100 g-^{1} DW

(Yagar et al., 2016). The afore-mentioned phenolic compounds depend on plant species, topographical location, genetic background, season, harvesting type, and parts of the plant (Šamec et al., 2017).

2.7 Problems Associated with Crop Residues

The use of crop residues is still minimal due to many reasons including storage issues, transportation problems, absence of agriculture extension services, lack of advanced technology, insufficient research trials and awareness at farmer levels (Lukuyu et al., 2011; Devendra and Leng, 2011; Anandan and Sampath, 2012; Loehr, 2012). Farmers do not have proper guidance to handle and store them. When they harvest, either they plow them with soil or burn them. Some crop residues and leaves of leguminous plants may cause metabolic disorders to animals like bloat (Wadhwa and Bakshi, 2013; Njidda, 2010). Most of the residues possess anti-nutritional factors (ANF). Some crops have mineral deficiencies, *i.e.* Brassica family is deficient of Iodine. It is a goitrogenic crop if iodine supplements will not be offered simultaneously. Sometimes, ruminants graze on turnip, tuber, bulbs, and maize cobs. These large pieces of food are stuck into the esophagus and block the digestive pathway. List of ANF in various feedstuffs and their effects on animals have been summarized in table 2.1.

Table 1.1. Various anti-nutritional factors reported in different feedstuffs

Feedstuffs	Anti-nutritional factors	Effects
Sorghum	Prussic acid, Tannin, Glycosides	Respiratory dysfunction, Bind with protein and stop the digestion
Soybean	Trypsin inhibitor, Lectins	Protein digestion impairment, Haemagglutinins
Brassica plants	Phenolics, Glucosinolates, Isothiocyanate	Fatty liver disease, Taint milk, Thyrotoxic, Goitrogenic, Poor growth
Vegetable leave	Nitrates, phytate, Glucosinolates, Phenolic content, Mineral deficiency	Disturbance in hemoglobin function, Chelate formation with minerals,
Rice and rice straw	Phytate, Lectins	Chelate formation with minerals, Haemagglutinins

2.8 Rumen Fermentation and Digestion in Ruminants

The stomach of ruminants consists of four compartments such as rumen, reticulum, abomasum, and omasum (Membrive et al., 2016). However, in suckling young ones, rumen and reticulum are comparatively underdeveloped and milk reaches directly to the abomasum and omasum through the esophageal or reticular groove (Martín-Alonso et al., 2019). As the young one starts eating solid food or a fibrous diet, the first two compartments generally called reticulo-rumen to develop greatly and become 85% of the total stomach in adult animals. The ingestion of high fibrous feedstuffs like straws and hay provides help in stimulating the development of reticulum. Whereas, the production of VFAs particularly butyric acid encourages the formation and enlargement of papillae, which increases the area of absorption for nutrients in the rumen. Consequently, both diets high in fibrous and starchy contents assist in developing rumen and weaning (Govil et al., 2017). The chewing process through teeth is adapted by ruminants for efficient grinding of a highly fibrous diet. The cheek teeth are large in size, resistant to wear, and provide an extensive grinding surface (Mansour et al., 2017). Ruminants produce a high amount of saliva during eating and rumination like 150 liters in cattle and 10 liters in sheep, which dilute the food (Gregorini et al., 2018). In the rumen, feed contents generally contain 85-93% moisture on average and exist in two phases; an upper phase having coarser solid material and a lower liquid phase with suspended fine food particles. The breakdown of the feed material is taken place through physical and chemical means. Ruminal contents are continuously mixed by rhythmic movements of rumen and rumination process. The factor which induces the rumination is a tactile stimulation of epithelium of the rumen, large particles of roughages, and some diets. The particle size of the roughage decides how much rumination will take (Membrive et al., 2016). Particularly, reticulo-rumen possess microflora such as anaerobic bacteria, protozoa, and fungi. Food is partially fermented by ruminal microflora and yielded to VFAs, microbial cells, gases such as methane, ammonia, and carbon dioxide. The gases are excreted through eructation and VFAs are absorbed through the rumen epithelial wall (Cabezas-Garcia et al., 2017). The microbial debris included partially degraded feed material pass to the abomasum and intestine with the flow of water and subjected to the process of digestion by enzymes. Digested material is absorbed through the intestine and available in the blood for cellular use. In the large intestine, VFAs also produce through microbial fermentation and absorb through the intestinal wall but undigested food along with microbial cells are excreted out in the feces (Vogel et al., 2017). Alike other isolated continuous culture systems, rumen also requires a homeostatic mechanism. Theoretically, VFAs production is capable of reducing ruminal pH up to 2.5-3.0 but pH is maintained at 5.5 to 6.5 due to the buffer activity of phosphate and bicarbonate present in saliva. Moreover, the rapid absorption of acids through the ruminal wall provides aid in maintaining pH. The flux of ion with each other maintains the osmotic pressure of rumen contents near to blood. Oxygen enters with food is rapidly consumed and anaerobiosis is maintained. Rumen liquor has a temperature (38–42°C) close to that of the animal. Finally, undegraded material along with microbial biomass and soluble nutrients move to the next part of the digestive tract through the reticulo-omasal orifice (Membrive et al., 2016).

In the rumen, over 200 bacterial species have been identified and the number can be 109-1010 per milliliter of rumen content. Most species are anaerobic and non-spore-forming. The relative population of specific species and the total number of bacteria vary and depend on the animal diet (Zhang et al., 2017). Concentrate enriched diet encourages total bacterial counts particularly lactobacilli. Over 100 species of protozoa have been identified but are less in number (106/mL) than bacteria in ruminal contents. However, being larger in size, it may equal the microbial mass. There are families of protozoa that exist in the rumen of adult animals. The isotrichidae (holotrichs) are ovoid in shape having cilia on the body. Whereas, the Ophryoscolecidae commonly called oligotrichs are vary in size shape, and appearance (Solomon et al., 2020). A normal ruminal microflora and microfauna are generally established in the early days of life in young ones (Martín-Alonso et al., 2019). The most unstudied microbes in rumen are anaerobic fungi and their role is yet to identify. They have a lifestyle as zoospore (motile phase) and sporangium (vegetative phase). During the vegetative phase, fungi penetrate the cell walls by attaching to food particles through rhizoids. They utilize polysaccharides and many soluble sugars however; mannose, pectin, arabinose, mannose, polygalacturonic acid, and galactose are not degraded by rumen fungi. The role of anaerobic fungi in the rumen has not yet been defined but their biomass becomes 10% of total microbial biomass when diets are rich in fibrous content (St-Pierre et al., 2018).

The ruminant diet generally contains soluble carbohydrates, starch, cellulose, and hemicellulose (Hatfield and Kalscheur, 2020). Young pastures contain 200g of soluble carbohydrates and 400g of cellulose and hemicellulose in each kilogram of DM. While, mature fodders like hay and straw can have higher cellulose, hemicellulose, and lignin contents but lower in water-soluble carbohydrates. All types of carbohydrates except lignin are subjected to attack by ruminal microbes. Major bacteria are involved from *Fibrobacter* and the *Ruminococci* genus, however, anaerobic fungi are also supposed to play a vital role in rumen fermentation (Owens and Basalan, 2016). The degradation process of carbohydrates involves two stages. In the first stage, the conversion of complex and bigger molecules to simple sugars occurs. This happens because of extracellular enzymes secreted by rumen microorganisms and this process is analogs to the digestion of carbohydrates occur in monogastric animals. The second stage involves the process similar to the metabolism of carbohydrates in the animal itself (Terry et al, 2019). Organic acids such as acetic acid, propionic acid, butyric acid, and gases are produced through carbohydrate digestion in the rumen. More as, fatty acids are also produced by the deamination of amino acids. The relative concentration of VFAs also varies with diet. Mature fodders containing high fibrous content produce a high proportion of acetic acid. However, less mature forages give rise to a lower concentration of acetic acid but a high proportion of propionic acid. The addition of concentrations into the ruminant diet also increases the proportion of propionic acid rather than acetic acid. Absorption of the acids produced by microbial degradation of carbohydrates occurs directly from the rumen wall. However, 10-20% part moves to the reticulum, omasum, and intestine and absorbs there (Murali et al., 2017).

Dietary proteins are degraded to peptides and amino acids by the action of ruminal microorganisms. However, some amino acids are further degraded into organic acids, ammonia, and carbon dioxide. The major bacterial species involved in proteolysis include *Prevotella ruminicola*, *Peptostreptococci* species, and the protozoa. The microbial proteins of rumen organisms are synthesized by utilizing ammonia, small peptides, and free amino acids. When rumen organisms are flushed to the abomasum and intestine their cell proteins are digested and absorbed. Moreover, bacteria have also the capability to synthesize the essential and non-essential amino acids making their host independent of dietary supplies of the former (Valente et al., 2016). The intermediate product between microbial degradation and protein synthesis is ammonia formation. When diet is protein deficient or resistant to degrade, the ammonia concentration in the rumen will be low and microbial growth becomes to slow down leads to retardation in carbohydrate degradation. Additionally, if protein degradation is rapid resulting in the accumulation of ammonia in rumen. Ammonia absorbs into the blood and converts to urea in the liver. Some urea returns to rumen via saliva and rumen wall through blood circulation but more part is wasted in the urine. The food supplied with less protein stimulates the return back of nitrogen from blood to rumen as urea which was absorbed as ammonia. The conversion of nitrogen into microbial protein depicts that protein reaching in the intestine is greater than that food (Gao et al., 2015). Primarily, each kilogram of DM yields 200g of protein. However immature forages yield into more protein-like 260g per each kilogram of DM. Counterwise, fewer ferment diets give a lower yield of microbial protein up to 130g/kg of organic matter in the rumen. However, additional protein can be synthesized from rumen bacteria by adding urea to the ruminant diet (Kertz, 2010).

Protein in food is not only the main component involves in ammonia production. Almost 30% of the nitrogen in the diet of ruminants includes simple organic compounds like amines, amides, amino acids, and inorganic compounds like nitrates. These non-protein nitrogen compounds are readily degradable in the rumen and convert into ammonia. Ruminal microflora converts them into protein. The most commonly used non-protein sources are urea, various derivatives of urea and salts of ammonia and urea may also be used (Carvalho et al., 2020). Urease enzyme hydrolyzes urea to ammonia, produced by ruminal microflora. Microbes convert ammonia into microbial protein but two conditions must be kept in mind. First, the initial concentration of ammonia should be lower than the optimum level. Second, the readily available energy source must be provided to microbes for protein synthesis. Therefore, urea mixed with such food having less rumen degradable protein and high fermentable carbohydrates should be provided (Nadeem et al., 2014). Avoid the animals from urea over-consumption, it absorbs rapidly from the rumen and overloads the capability of the liver to reconvert it to urea, leading to a high concentration of ammonia in the blood, becomes a serious cause of ammonia toxicity (Patra, 2015).

The dietary lipids of ruminants contain a high proportion of linoleic acid, linolenic acid, and polyunsaturated acids (Anthony et al., 2000). These triacylglycerols are hydrolyzed by ruminal microbes and convert them into phospholipids. The complex and polyunsaturated fatty acids are hydrogenated by microorganisms yielding into simpler ones like monoenoic acid and stearic acid. The *trans* acids can be found commonly in rumen contents because *cis* double bonds of linoleic and linolenic acids convert into *trans* configuration due to hydrogenation (Ribeiro Alves Lourenço et al., 2010). The ruminal microflora also synthesizes a significant amount of lipids, which become part of milk and body fats (Kalač and Samková 2010). Lipid degradation in the rumen is limited. The increased amount of lipid decreases the activities of rumen microbes leads to the retardation of fiber digestibility and feed intake. Unsaturated fatty acids affect less rumen fermentation as compared to saturated ones. Rumen fermentation is little effected by calcium salts of fatty acids thus their incorporation as fat supplements in the ruminant diet could be a viable option. Long-chain fatty acids do not directly absorb from the rumen wall. They become saturated and unesterified on entering the small intestine. Mixed micelles are formed with the help of lysophosphatidylcholine in ruminants, which provide aid in absorption (Brzozowska and Oprządek, 2016). In ruminants, predominantly stearic acid (synthesized in the rumen from hydrogenation) present in the composition of body fats. However, modification of the composition of body (milk and meat) fats is possible, if dietary lipids (unsaturated fatty acids) are treated in such a way that they are protected from the biohydrogenation caused by microorganisms in the rumen (Das et al., 2019).

The amount of vitamin B complex production becomes relatively small when ruminants receive foods enriched with B vitamins. If the diet is insufficient, rumen microbes increase the production of vitamins. Ruminal microbes increase the production of vitamin intake in the diet decreases (Xie et al., 2019). Therefore, adult ruminants are independent of dietary supplementation of vitamins but for the synthesis of vitamin B12, a sufficient supply of cobalt must be ensured (Lei et al., 2019).

2.9 Previous Research on Utilization of Crop Residues for Livestock

Inadequate quality and quantity of feed for ruminants during the lean periods (extreme summer and winter) is a major challenge for efficient livestock production. Globally, a large quantity of crop residues is produced, which possess good nutrient profile, alternatives of conventional feedstuffs during feed shortage periods. Generally, farmers either burn the crop waste and residues in the field or plow them into the soil. The environment is being compromised due to the burning issues of excessive crop waste in recent years. Thus, the production of vegetables and fruits is constantly growing, making byproducts and waste abundantly available. These resources could be used as potential feed for animals especially in ruminant nutrition. There is a need for time to explore the ways and options to improve the nutrient values of crop residues and utilize them in more effective ways.

For the preservation of crop residues especially brassica plants, Rezende et al. (2015) conducted an experimental study to preserve the cabbage in the form of silage that is treated with 400 and 600 g/kg of ground corn. Results showed that 400 g/kg ground corn was enough for improvement in fermentation properties of cabbage silage, whereas the use of bacterial inoculant is not recommended. Similarly, Ren et al. (2018) conducted a study to check the effects of cellulase enzyme on the quality parameters of cabbage and corn mixed silage. They revealed that 0.1% of cellulose improved the quality and fermentation of silage. The pH and ammonia nitrogen were decreased while lactic acid and water-soluble carbohydrates were significantly increased. These studies show that preservation of crop residues is possible and can improve their nutrient profile and quality.

In addition, crop wastes and residues have various effects on animal production. Most of the time, they improve the growth, performance, and production of animals. Some experiments showed that they are efficiently digestible by ruminants. Researchers have also determined their nutritional qualities as feed. In a study, broccoli was used as a substitute for concentrates in dairy cattle. There were no significant results found on milk protein, lactose, and milk components. However, milk fat was increased significantly. It showed that broccoli can be included in the diet of dairy cattle at an appropriate level to replace concentrates without posing any harmful effect (Yi et al., 2015).

Differently, Ngu and Ledin (2005) evaluated the effects of feeding cabbage leaves on FI, bodyweight in goats. Low FI was observed in goats due to less fibrous content. However, weight gain was increased.

Similarly, Megersa et al. (2013) investigated the effects of sweet potato leaves to replace concentrate on nutrient digestibility, growth performance parameters, and carcass properties of bucks. Results revealed that DM intake, CP intake, DM digestibility, and weight gain increased due to supplementation of sweet potato leaves in the diet. The carcass characteristics were higher in supplemented goats compared to the unsupplemented. Sweet potato could replace conventional feedstuffs like poor hay and concentrates on preventing animal weight loss.

Pimentel et al. (2017) conducted a study to check the effects of dietary supplementation of sun-dried banana peel for Holstein x Zebu cows on intake, digestibility, and milk production. Results showed that dry matter intake was increased with a maximum level at 38.30% substitution of sorghum silage. While CP intake, and NDF digestibility were decreased. Variation in growth performance and production parameters like weight and body condition score, milk production, and feed conversion were not affected. Findings show that banana peel can be replaced with sorghum silage without affecting animal production.

Monção et al. (2016) checked the digestibility of banana peel treated with 1, 2, 3, and 4% of limestone and dried in the sun for 3 days. There was no effect observed on DM and NDF degradability. Thus, banana peel treatment with limestone is not recommended as an additive. Similarly, Ramdani et al. (2019) investigated the potential of banana peel for ovine feeding. Proximate chemical analyses revealed that raw banana peel had higher DM, TDN, CF, and GE than ripened banana peels. *In vitro* digestibility trials showed that raw Ambon banana peels had lower DM digestibility, OM digestibility, and VFA, while NH3 was higher than ripened peels. Both raw and ripened banana peels could be used as a potential source of sheep feeding as their dietary supplementation up to 10-40% replacing roughages can increase DM digestibility, OM digestibility, VFA, and decrease NH3.

The nutrient profile of brassica vegetables particularly cabbage and Brussels sprouts also show a high source of CP and carbohydrates for ruminants. For the determination of nutritional value and digestion of cabbage and Brussels sprouts, Eván et al. (2018) conducted an experiment. DM was observed ranged from 6% in cabbage to 16% in Brussels sprouts. While, cabbage possessed higher CP, EE, NDF, and ADF than

Brussels sprouts. Cabbage had higher ammonia nitrogen concentration than Brussel sprouts due to higher CP contents. Both vegetables have a good source of protein and carbohydrates indicating their potential use as ruminant feed and extensively fermented *in vivo* and *in vitro*.

Similarly, Hossain et al. (2016) also conducted a study to evaluate the nutrient composition of different vegetable waste and leaves to utilize them as a potential non-conventional source for ruminant feeding to reduce feeding cost. The CP content in banana tree was 15.6%, bean leaf 28.2%, bilimbi leaf 11.9%, cabbage 18.9%, cauliflower 17.3%, pumpkin leaf 25%, radish 14.9% and spinach 12.9%. Moreover, all vegetables consisted of a significant amount of CF, NFE, EE, and ash contents. It is therefore concluded that vegetable waste could be used in a significant amount as alternatives to conventional feedstuff for animals.

Researchers have determined the nutritional value of various feedstuff especially crop residues through *in vivo* and *in vitro* methods. Meneses et al. (2020) evaluated the ensiled artichoke and boiled broccoli for *in vitro* rumen degradability and *in vivo* digestibility. Both silages showed high disappearance in the in-vitro DM degradability trial. While artichoke and broccoli were 78.5% and 80.0% *in vivo* digestible, respectively. Whereas, CP and NDF digestibilities were found high in boiled broccoli silage. Nutritive values and digestibilities of these silages indicate that both can be used for feeding ruminants and are an environmentally friendly way of disposal of such residues.

Song et al. (2020) conducted a study to examine the chemical composition and in situ digestibility of Chinese cabbage, cabbage and vegetable-fruit by-products and their feeding effects on the performance of growing Hanwoo steers. The CP contents determined in Chinese cabbage, cabbage and vegetable-fruit byproducts were 20.20%, 18.69%, and 10.07%, respectively. NDF was found higher in cabbage followed by Chinese cabbage and fruit-vegetables. The vegetable-fruit byproducts (DM 84.69%; NDF 85.62%) were degraded more as compared to cabbage (DM 68.47%; NDF 55.97%) and Chinese cabbage (DM 68.09%; NDF 54.22%). Animal weight, growth, and FCR were not influenced by the inclusion of this waste and byproducts in the diet of animals.

Likewise, nutrient composition, *in vitro* ruminal fermentation, and intestinal digestibility of discarded cabbage, and Brussels sprouts were evaluated by Eván et al. (2019). All cabbages contained DM up to 17% while their CP and sugar contents ranged from 19.5-24.8% and 27.2-41.4%, respectively. However, due to low NDF contents (17.5-28%), they were rapidly degraded in the rumen.

El-Shinnawy et al. (2011) designed a study to improve and utilize the cabbage waste as an unconventional feed source for ruminant animals in the form of hay and silage treated with urea. Clover hay was replaced by cabbage wastes as hay or silage using simple technologies for improving the nutritive value and proper use of cabbage wastes as ruminant feed. Fermentation characteristics of silages revealed that cabbage wastes silages were excellent, had a normal value of pH (3.82 to 4.12) with the superiority of unureated silage. TVFA for two silages were ranged from 15% for unureated silage to 2.45% for ureated silage. The unureated silage recorded the least concentration of NH3-N. Silages either treated with or without urea showed high digestibility of OM, CP, CF, NFE, NDF, ADF, and cellulose and improved significantly TDN, DCP, and nitrogen balance values. The rams fed cabbage silage had lower ruminal NH3-N than other rations. TVFA pattern followed that reverse trend of NH3-N. The ensiling process increased the DM intake in comparison with other rations containing cabbage wastes hay. Using cabbage wastes in the form of hay or silage as well as improvements in their nutritional composition through urea may act as good unconventional feedstuffs for ruminants and decrease the feed cost, equivalent to any conventional feed like clover hay.

2.10 *In vivo* Digestibility Trials

Digestibility in animal nutrition defines as the percentage of feed and its nutrients which are digested, absorbed, and used by body cells (Schneider and Flatt, 1975). There are two methods to measure the digestibility of various feedstuffs include *in vivo* techniques by using animals and *in vitro* techniques through laboratory methods. However, *in vivo* digestibility does not maintain feed characteristics, setting a limit of accuracy to which *in vivo* digestibility can be predicted (Tilley and Terry, 1963). This method has also known to be high priced, laborious, time-consuming, and difficult to manage large animals as well within allocated confined places (Earing et al., 2010). Consequently, *in vitro* fermentation techniques are gaining popularity among researchers in ruminant nutrition to study digestion and ruminal fermentation characteristics (Lattimer et al., 2007). Conventionally, the *in vivo* digestibility technique was used as a standard for evaluating the quality of forages. Before starting the experiment, the feed must be prepared. The chemical composition of feed should be determined using proximate analysis. Digestibility trials contain three phases. Animals are moved to a test diet in the first phase. In the second phase, animals become acclimatized to test diets to ensure that previous diets are removed from the digestive tract. After this, animals must be placed in confined spaces and adjusted to the diet being studied to allow for rumen microflora to adapt (Schneider and Flatt, 1975). Following the adaption period, feed intake and out is recorded regularly, and feed intake must be constant thought the digestion period to avoid any error in digestibility measurements. In the collection phase, the total fecal output must be collected in bags attached to the back of animals, via metabolic crates or from clean ground surfaces (Schneider and Flatt, 1975). Feces and orts may be collected as 10% of the whole collection for each day and pooled with each other and stored at -20C until chemical analysis will be performed. For analyzing amounts of water, DM, CP, CF, NDF, ADF, ADL, OM, and ash, thaw the frozen samples, and put them into hot air oven at 60 for 48 hours. Later, samples must be ground with grinding mill machines and prepared for proximate analysis. The difference between feed intake and feed excreted in feces is termed as *in vivo* apparent digestibility (Schneider and Flatt, 1975). True digestibility excludes endogenous sources of nutrients and represents only the portion of nutrients absorbed from the digestive tract (Pond et al., 2004).

2.11 *In vitro* Digestibility Trials

In vitro digestibility methods have been applied successfully in swine, human, and other monogastric animal research to predict the *in vivo* behavior of single or multiple nutrients using different methods. Tilly and Terry (1963) developed an *in vitro* digestibility method to forecast the *in vivo* digestibility pattern of nutrients using single or multistep procedures (Earing et al., 2010). However, this technique has some drawbacks as ruminal/cecal fluid is used as an inoculum source, cannulated animals are not readily available all the time while experimenting (Earing et al., 2010). Consequently, rumen liquor from slaughter animals and/or feces is an alternatives inoculum source because ruminal microflora can remain effective and useful several hours after collection (Earing et al., 2010). The experimental diet is degraded in the specific inoculum (cecal fluid, rumen fluid, or feces) and buffer solutions for a specific period of time to stimulate the microbial fermentation (Tilley and Terry, 1963). Then, either *in vitro* DM digestibility or two-stage dry matter disappearance methods can be used for the estimation of nutrient digestibility of forages. In two-stage DM disappearance, feed samples are degraded in either pepsin or a neutral detergent solution after microbial fermentation (Tilley and Terry, 1963; Van Soest et al., 1966). The three-stage batch method is the most common method for studying the fermentation and digestion of nutrients. The first two stages are autoenzymatic including stomach and intestine while the third contains alloenzymatic including hindgut. It is simpler than a continuous system because of no input and output batch system, which is mostly used for studying the microbial ecology of the large intestine (Coles et al., 2005). Meyer et al. (1971) observed that Tilley and Terry (1963) and NDF methods to be superior techniques, further indicated that digestion of feed samples beyond ruminal fermentation is necessary to determine the *in vivo* forage digestibility. Over the years, some modifications have been made by many laboratories in Tilley and Terry (1963) method to suit their needs and laboratory situations such as the development of the Ankom Daisy^{II} incubator (Ankom Technology, Macedon, NY). Wilman and Adesogan (2000) compared the Tilley and Terry method by Ankom Daisy^{II} incubator with a conventional two-stage method using tubes to measure the *in vitro* digestibility of forages. The digestibility of a combination of two forage species was measured using rumen liquor obtained from both sheep and cattle. For the filter bag method, Ankom F57 filter bags (Ankom Technology, Macedon, NY, USA) and the Ankom Daisy^{II} incubator were used. For Ankom technology, 250-500 mg of feed sample in each bag and a total of 25 bags in one incubation jar, both high and low digestibility standards and a blank are recommended. While in other experiments, tubes of 100 mL made of polypropylene were used for true digestibility and apparent digestibility. For digestibility, 21 tubes containing 500 mg of feed sample with standards like Ankom technology were used for apparent and true digestibilities. The same rumen fluid/buffer mixture, acid pepsin solution, neutral detergent solution, and incubation temperature (39°C) were used for filter bag and tube methods. The estimation of true digestibility was found lower in Ankom technology than tube method while apparent digestibility was found high using the filter bag method than tubes. Results showed that the conventional method was proved more precise however filter bag also provided acceptable results and required less time and labor. Whereas, Tilley and Talley (1963) method is laborious, expensive, and time-consuming and requires each feed to be incubated independently, makes Ankom daisy^{II} incubator a perfect and efficient method in measuring *in vitro* DM digestibility (Lattimer et al., 2007). Several factors influence the *in vitro* DM digestibility. One of the major factors is inoculum, which depends upon the animal species and type of diet fed to donor animals. The additional variation could be the particle size of the experimental diet, the weight of the substrate, ration of inoculum to buffer, and length of time for the experimental procedure.

CHAPTER III

MATERIALS AND METHODS

In vivo apparent nutrient digestibility trials were conducted at Ayhan Şahenk Agricultural Research and Application Center, Niğde Ömer Halisdemir University Turkey from 15 January to 17 March 2020. *In vitro* true digestibility trials were carried out at Animal Production and Technologies, Niğde Ömer Halisdemir University Niğde Turkey from 25 August 2020 to 11 September 2020.

3.1 *In vivo* Apparent Nutrient Digestibility Trials

3.1.1 Ensiling process

Preservation of high moisture crops under pressure and anaerobic conditions due to the fermentation of water-soluble carbohydrates are called the ensiling process. The fermentation process lowers the pH of silage within the range of 3.8-4.2. VFAs such as acetic acid, butyric acid, and propionic acid, and other organic acids are produced in the result of fermentation and inhibit the proteolytic activities of various bacteria like Clostridia genus and preserve the crops efficiently. For silage preparation, fresh vegetable leaves of cabbage and kohlrabi were collected from the nearby village in September 2019 and transported to the Ayhan Şahenk Agricultural Research and Application Center through the truck. The collection was performed during the morning time till noon. The leaves were weighed properly and spread uniformly on the concrete floor for wilting. The ambient temperature was up to 30^{o}C or above when wilting. The leaves were left to dry in the sun for 3-5 days to reach a moisture level which was good for the ensiling process. Later, leaves of cabbage and kohlrabi were chopped with an automatic chopper up to 5-10 cm length. Samples collection was done for chemical characterization of the kohlrabi and cabbage before ensiling. Two silages were made; cabbage leaves silage (CS) and cabbage + kohlrabi leaves silage (CKS). In CKS, 50% cabbage and 50% kohlrabi leaves were mixed. Silages were pressed with the tractor and covered with a double layer of polyethylene sheet, equipped with clamps and weighted down with heavy bricks, tires, and hay bales. The compacted leaves lasted for 120 days. After three months of ensiling, the polyethylene sheet was uncovered from one corner and duplet samples were collected before diet formulation. The samples were analyzed for physical, chemical (DM, OM, CP, NDF, ADF, and ash), and fermentative characteristics (pH). After the silo was opened, silages were continuously taken off to feed animals using a daily silage extraction slice of 10-15cm for consecutive 60 days from each silo.

3.1.2 Diet Plan

Silages of two vegetable leaves (cabbage and kohlrabi) were used in the feeding trial. Three diets were prepared using three feedstuffs. One diet consisted of single feed ingredient alfalfa hay (100%). The other two diets were comprised of alfalfa hay with CS (50:50) and alfalfa hay with CKS (50:50). Chemical composition of rations which were used in *in vivo* apparent digestibility trials is given in table 3.1.

- Ration 1: Alfalfa hay (AAH) as control with the ratio of 100
- Ration 2: CS + AHH with the ratio of 50:50
- Ration 3: CKS + AHH with the ratio of 50:50

Table 2.1. Chemical composition of rations used for *in vivo* apparent nutrient digestibility trials

Items	R1	R2	R3
DM	81.1	49.2	54.2
OM	88.7	81.4	82.7
CP	16.5	16.4	17.7
NDF	41.5	33.8	32.1
ADF	31.2	23.8	22.9
Ash	11.3	18.1	17.4

R1= alfalfa hay (100), R2= alfalfa hay and cabbage silage (50:50), R3= alfalfa hay and cabbage + kohlrabi silage (50:25:25), DM = dry matter, OM = organic matter, CP = crude protein, NDF = acid detergent fiber, ADF = acid detergent fiber

The reason for adding alfalfa hay in diets was to protect the animals from any digestion upsets because of chopped silage. Brassica plants are highly fermentable in the rumen and have a high amount of soluble carbohydrates with less NDF. Therefore, it was necessary to add high fibrous feedstuff like alfalfa hay with silages to avoid metabolic disorders in animals. Rams were fed to cover maintenance requirements (NRC, 2007) and the total DM offered to rams was according to the maintenance level.

3.1.3 Animals, housing, and experimental design

For estimation of apparent nutrient digestibility of the alfalfa hay with cabbage and cabbage + kohlrabi leaves mix silages; three Akkaraman rams of 80 ± 7.3 kg body weight were used. Animals were placed in separate stalls. The stalls had head gates and plastic grid floors which allow the urine and feces to go down to the cemented floor. Animals were randomly assigned to one of the three experimental diets using 3 × 3 Latin square design. The total experimental period lasted for 60 days. Each digestion period was carried out with three rams and lasted for 20 days of which 15 days were the adaptation period followed by a collection period of 5 days. The experimental diets were 100% AAH, 50% AAH with 50% CS and 50% AAH with 50% CKS. Within each Latin square, rams were allocated to each of the three treatments randomly at the start of the experiment. Animals were allowed to adjust to metabolism crates for 3 days prior to the start of the experiment. Each stall was equipped with a water bucket and a manual feeder. Freshwater was available ad-libitum in front of the animals at all times. Daily FI was recorded. The cleaning of the floor was done at 0800 and 1700 hours each day of the experimental period. Animals were fed twice a day, as equal parts at 0800 and 1700 hours. Animals were weighed in the first and last days of each digestion period. Feed leftovers were collected each day during the collection period in the morning at 0800 hours. Ram was fitted with fecal collection bags to the back of each ram three days before the collection period started. Bags were emptied and feces weighed and sub-sampled twice daily and feces were collected twice a day in the morning 0800 and the evening 1630 hours before the next ration was provided. The feed leftover and feces were weighed and subsamples as 10% of total weight and stored at -20^{o}C in the deep freeze until chemical analyses were done. Later, feces and ort samples of each ram were thawed and 5-day cumulative samples were pooled by mixing daily samples. All the samples were dried for 2 days in a forced-draft oven at 65^{o}C to a constant weight and ground in a 1-mm screen laboratory mills (Retsch ZM 200) for further determination of nutrient composition and apparent nutrient digestibility. The apparent nutrient digestibility coefficients of the diets were determined through the direct method (total collection method) according to the given formula (3.1). Moreover, test feed (cabbage silage and cabbage + kohlrabi silage) digestibility from the mixed diets was also calculated using the difference technique equation (3.2).

$$\text{In vivo Digestibility} = \frac{\text{Nutrient intake} - \text{Nutrient excrete}}{\text{Nutrient intake}} \times 100 \qquad (3.1)$$

$$\text{Nutrient digestibility in test feed } = \frac{(\text{A}) - (\text{B})(\text{C}) \times 100}{\text{D}} \qquad (3.2)$$

A = Nutrient digestibility in the total diet
B = Nutrient digestibility in the basal diet (determined when fed alone)
C = Proportion of total nutrient in diet supplied by basal diet
D = Proportion of total nutrient in diet supplied by test feed

3.2*In vitro* True Digestibility using the Ankom Daisy^{II} Incubator

3.2.1 Apparatus

In vitro true digestibility (IVTD), OM digestibility, and NDF digestibility were performed using Ankom Daisy^{II} Incubator including various apparatuses like filtration device, F57 filter bags, heat sealer, and thermos. Daisy incubator was used as the main fermenter chamber. The thermos was used to carry rumen liquor from the nearby slaughterhouse at 39^{o}C. The substrate was put in filter bags after weight measuring and sealed with heat sealer.

3.2.2 Feed sample preparation

Cabbage silage, cabbage + kohlrabi silage, fresh cabbage leaves, and fresh kohlrabi leaves were used to determine *in vitro* digestibility coefficients using Ankom Daisy^{II} Incubator (table 4.1 and table 4.2). The samples were dried in an air-forced oven for 48 h at 50°C and ground to pass through a 1 mm screen. Prior to putting feed samples, Ankom F57 filter bags were rinsed with acetone (99.5%) for 3 minutes and then dried, labeled, and weighed. The bags were filled with 500 mg of ground samples and heat sealed. Moreover, empty filter bags were sealed and served as a blank for calculation of the correction factor. Three batches were performed for this experiment and each feed was run in triplicate in each batch.

3.2.3 Procedure

IVTD of feed ingredients were measured by using the Daisy^{II} Incubator according to the methodology described in Ankom Technology (Macedon, NY). The Daisy^{II} Incubator consists 4 incubation vessels having a capacity of 2000 mL each. 1600 mL buffer solutions and 400 mL rumen liquor with 25 filter bags can be added in each vessel. The rumen fluid was collected postmortem from the rumens of two mature Holstein cattle that were slaughtered in a commercial abattoir in Niğde. Two thermoses were used to store and transport the rumen fluid, which were preheated to 39°C and had CO_2. The rumen fluid was collected manually by squeezing two handfuls of ruminal contents from each animal's rumen into the same thermos. The thermoses were transported to the laboratory at 39^{o}C just after the rumen liquor collection. In the laboratory, the rumen fluid was filtered through 4 layers of cheesecloth into a five-liter flask (pre-heated 39° C). The buffer solutions to be used in the analysis were prepared before one night according to the Ankom Daisy *in vitro* fermentation system described in Ankom. Buffers consisted of 2 solutions that were combined in the incubation jars immediately before the rumen inoculum. Buffer A and B were mixed in a way to obtain a final pH of 6.8. Chemical used for buffer A and B are given in table 3.2.

Table 3.2. Buffer A and B solutions for *in vitro* digestibility trials using Ankom Daisy^{II} Incubator

Chemicals	g/liter
Buffer Solution A	
KH_2PO_4	10.0
$MgSO_4•7H_2O$	0.5
NaCl	0.5
$CaCl_2•2H_2O$	0.1
Urea	0.5
Buffer Solution B	
$Na2CO_3$	15.0
$Na_2S•9H_2O$	1.0

Four digestion units were used in this test. The final buffer solution was heated to 39°C and 1600 mL was poured into each digestion unit. Filter bags containing feed samples were put into incubation vessels and later 400 mL of rumen fluid was added to each. The F57 filter bags containing experimental diets were placed digestion vessels and

CO_2 was purged for thirty seconds and then sealed. The sealed digestion vessels were placed in the pre-warmed unit and incubated for 48 hours at 39 ± 0.5°C with continuous agitation. The filter bags were washed under running water after 48 hours of incubation. The bags were rinsed thoroughly with cold tap water until water is clear to stop the microbial activity. Care was taken to use minimal mechanical agitation during rinsing. Bags were stored in the refrigerator until NDF was not determined. Later, bags were placed in the Ankom^{200} fiber analyzer and follow the procedure for determining NDF. The post *in vitro* NDF weight was recorded as W3. The IVTD as fed and DM based was calculated using the following formula (3.3 and 3.4). *In vitro* OM digestibility was measured according to following equation (3.5). Moreover, *in vitro* NDF digestibility was determined using given formula (3.6).

$$\mathrm{IVTD\ (As\ fed)} = \frac{100 - (\mathrm{W3} - (\mathrm{W1} \times \mathrm{C1})}{\mathrm{W2}} \times 100 \tag{3.3}$$

$$\mathrm{IVTD\ (DM)} = \frac{100 - (\mathrm{W3} - (\mathrm{W1} \times \mathrm{C1})}{\mathrm{W2}\ \times \mathrm{DM}} \times 100 \tag{3.4}$$

$$\mathrm{IVOMD} = \frac{100 - (\mathrm{W3} - (\mathrm{W1} \times \mathrm{C1})}{\mathrm{W2}\ \times \mathrm{OM}} \times 100 \tag{3.5}$$

W1 = Bag tare weight
W2 = Sample weight
W3 = Final bag weight after In vitro and sequential NDF treatment
C1 = Blank bag correction (final oven-dried weight/original blank bag weight)

$$\mathrm{NDF\ digestibility}\ = \frac{\mathrm{NDF0h} - \mathrm{NDF48h}}{\mathrm{NDF0h}} \times 100 \tag{3.6}$$

NDF_{0h} = NDF content in feed before incubation
NDF_{48h} = NDF content in feed after incubation

3.3 Analytical Procedures

DM, OM, ash, and CP were determined according to procedures described by AOAC (2000). NDF and ADF determination was done in F57 filter bags using (Ankom

Technology Corp., Macedon, NY). Moreover, total phenolic contents were analyzed from fresh cabbage and kohlrabi leaves following the Folin-Ciocalteu method (Liang et al., 2019). The antioxidant capacity of cabbage and kohlrabi leaves was determined using the Trolox Equivalent Antioxidant Capacity method (Managa et al., 2020).

3.3.1 Dry matter measurement

For the determination of DM, 1 g of sample was taken in a clean crucible. Crucibles were put into hot air oven for 8 hours at 105^{o}C. After a given time, crucibles were transferred to a desiccator to let them cool to room temperature. Following this, crucibles were reweighed as quickly as possible to prevent moisture absorption. DM percentage was measured using the given formula (3.7).

$$\text{Dry matter (\%)} = \frac{\text{W1} - \text{W2}}{\text{W1}} \times 100 \tag{3.7}$$

W1 = weight of the sample before drying
W2 = weight of the sample after drying

3.3.2 Crude ash and organic matter estimation

Crude ash estimation was done using a muffle furnace at 600^{o}C for 4 hours. One gram of sample was taken into clean dried (105^{o}C for 8 hours) crucibles and placed them into the muffle furnace. After burning, the crucibles were transferred into desiccator to let them cool. Crucibles were reweighed and crude ash and OM was calculated using the following formula (3.8). However, OM was determined by subtracting crude ash % from 100.

$$\text{Crude ash (\%)} = \frac{\text{Weight of sample after ashing}}{\text{Weight of Sample}} \times 100 \tag{3.8}$$

3.3.3 Crude protein estimation

Crude protein was estimated using KjelROC Digestion Systems and the KjelROC Analyzer (Opsis LiquidLine, Sweden). One gram of each sample were put in thetubes and 12 mL of sulfuric acid and 2 Kjeldahl tablets (97% Potassium sulfate and 3% Copper sulfate hydrate) were added. Samples were digested in a preheated KjelROC Digestion System with a temperature of 420°C. The digester switched off and let the tubes cool after 60min of digestion. Tanks of distil water, 40% sodium hydroxide solution, and 1% boric acid with mixed indicators (100 mg bromocresol green and 70 mg methyl red solved separately in 100 mL methanol) were attached to KjelROC Analyzer (Distillation and titration unit). The program was set in the analyzer to measure the crude protein values.

Crude protein = total nitrogen × 6.25

3.3.4 Neutral detergent fiber estimation

For the determination of NDF, Ankom fiber analyzer 200 was used. For the preparation of NDF solution, 30g Sodium dodecyl sulfate, 18.61g Ethylenediaminetetraacetic disodium salt, 6.81g Sodium borate, 4.56g Sodium phosphate dibasic (anhydrous), and 10.0 mL Triethylene glycol was mixed to 1 L distilled H2O. Moreover, alpha-amylase and Sodium Sulfite were used. Empty filter bags were weighed and record the weight as (W1). For each sample, 500 mg was placed in empty filter bags and noted the weight as (W2). An empty bag was included to determine the blank bag correction (C1). Bags were closed using a heat sealer. Samples were spread uniformly inside the filter bags to eliminate clumping. Filter bags were placed on bag suspender trays and trays were inserted into the vessel. Hot water supply was confirmed and then added 2 L of neutral detergent solution, 20g of Na_2SO_3, and 4.0 mL of alpha-amylase. The whole procedure of NDF was 75 minutes. Washing of filter bags inside fiber analyzer was done with two times hot water including 4 mL alpha-amylase and two times with simple hot water and cold water. After the NDF extraction and rinsing procedure, the vessel was opened filter bags were removed to squeeze excess water. Later, bags were placed into a 250mL beaker of acetone to cover bags and soaked for 3-5 min. After that, the filter bags were removed from acetone and let them dry of acetone. Thereafter the bags were put into the oven for 2 hours at 105°C. Bags were removed from the oven and weight was measured and record as (W3). NDF (%) was determined by using given formula (3.9).

$$\text{NDF } (\%) = 100 \times \frac{\text{W3} - (\text{W1} \times \text{C1})}{\text{W2}} \tag{3.9}$$

W1 = bag tare weight

W2 = Sample weight

W3 = Dried weight of bag after extraction process

C1 = Blank bag correction

3.3.5 Acid detergent fiber estimation

For the determination of ADF, Ankom fiber analyzer 200 was used. For the preparation of the ADF solution, 20 g cetyltrimethylammonium bromide was mixed with 1L of 1.00N H_2SO_4. Empty filter bags were weighed and record the weight as (W1). For each sample, 500 mg was placed in empty filter bags and noted the weight as (W2). An empty bag was included to determine the blank bag correction (C1). Bags were closed using a heat sealer. Samples were spread uniformly inside the filter bags to eliminate clumping. Filter bags were placed on bag suspender trays and trays were inserted into the vessel. Hot water supply was confirmed and then added 2 L of acid detergent solution and press the start button following the ADF program. Following this, pushed the start button and close the vessel lid. The whole procedure for ADF was 60 minutes. Four washing was done inside fiber analyzer after process with three times hot water and one time with cold water. After the ADF extraction and rinsing procedure, the vessel was opened filter bags were removed to squeeze excess water. Later, bags were placed into a 250 mL beaker of acetone to cover bags and soaked for 3-5 min. After that, the filter bags were removed from acetone and let them dry of acetone. Thereafter the bags were put into the oven for 2 hours at 105^{o}C. Bags were removed from the oven and weight was measured and record as (W3). ADF (%) was measured by using following formula (3.10).

$$\text{ADF } (\%) = 100 \times \frac{\text{W3} - (\text{W1} \times \text{C1})}{\text{W2}} \tag{3.10}$$

W1 = bag tare weight

W2 = Sample weight

W3 = Dried weight of bag after extraction process

C1 = Blank bag correction

3.3.6 Silage pH

Fifty grams of fresh silage with 450 mL of distilled water was blended in laboratory blender for 5 minutes to obtain silage extract (Tjardes at al., 2000). Silage extract was filtered through 4 layers of cheesecloth. Later pH of silage extract was measured by a laboratory pH meter (Mettler-Toledo, USA).

3.3.7 Total phenolic content of cabbage and kohlrabi leaves and their silages

The determination of TPC is based on a redox reaction. Phenolic compounds in samples react with Folin-Ciocalteu reagent and turn it into an oxidized form. Gallic acid is used as a standard for the preparation of the standard graph. Different concentrations of Gallic acid are used with ethanol (1-0.5-0.25-0.125-0.0625-0.03125 mg/mL) and their absorbance is read. The graph of absorbance is plotted against concentration. TPC are estimated following the graph (Slinkard and Singleton, 1977). For evaluation of TPC, extracts were obtained from fine powders of experimental diets and their silages. For this, 20 g of each sample were used and mixed with 200 mL of 80% ethanol. The samples were kept on an electric stirrer for 24 hours at ambient temperature for proper mixing. The extracts were then filtered through Whatman No^{o}4 filter paper. Supernatants combined with ethanol were evaporated under vacuum at 45C^{o} in a rotary evaporator. The remaining liquids were stored at -80C^{o} for the evaluation of total phenolic content through the Folin-Ciocalteu method. For the preparation of Folin-Ciocalteu reagent, 5 mL of 0.2 N Folin-Ciocalteu reagent was mixed with 900 µL of distilled water. Further 4 mL of saturated sodium carbonate (Na_2CO_3) solution (7.5 g/L) was added to 100 µL of the solution diluted from the vegetable leaves extract. After this, samples were kept under dark for two hours, and then their absorbance was read against the curve in the spectrophotometer at 765 nm. Total phenolic contents were determined as microgram Gallic acid equivalent per 100 g of dry weight using an equation obtained from standard Gallic acid graph.

3.3.8 Antioxidant activity of fresh cabbage and kohlrabi leaves

Antioxidant capacity is measured with Trolox Equivalent Antioxidant Capacity (TEAC) analysis. It is based on the inhibition of the absorbance of the 2,2'-azinobis 3-ethyl-bezothiazoline 6 sulfonate (ABTS) radical cation by antioxidants. For this purpose, 7 mM ABTS solution containing 2.45 mM potassium persulfate was prepared. A radical solution was formed by storing ABTS solution for 12-16 hours at room temperature in the dark place. Series of extract concentrations and Trolox were prepared for the determination of antioxidant activity. 10 µL of each sample was mixed with 1 mL of ABTS solution and a decrease in absorbance was observed for 6 minutes through spectrophotometers. The slop was calculated from the graphs where percent inhibition was plotted against the concentrations. The antioxidant activity was measured by the slop ratio of vegetable extracts to the Trolox slop concentrations mentioned as 1 mM Trolox response. Example slope/slope of Trolox) x dilution factor = TEAC value µM Trolox.

3.4. Statistical Analyses

Data were analyzed using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). *In vivo* apparent nutrient digestibility trials were conducted using 3 × 3 Latin square design. All the values presented in this thesis as the mean ± standard error of mean (SEM), and $P<0.05$ was considered statistically significant. One way analysis of variance (ANOVA) and Tukey’s Multiple Comparison test were used for comparing the control and treatment groups.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Chemical Composition of Fresh Cabbage and Kohlrabi Leaves

The chemical composition (% of DM) of fresh cabbage and kohlrabi leaves is shown in table 4.1. As expected, cabbage and kohlrabi leaves had low DM content ranging from 11.42 to 16.7%, respectively. Fresh kohlrabi leaves had greater DM (16.7 ± 0.9 %) but lower OM (79.5 ± 0.8 %) as compared to fresh cabbage leaves DM (11.42 ± 1.3 %) and OM (81.3 ± 0.7 %). The DM of kohlrabi leaves was comparatively rich in protein. Crude protein was found highest in fresh kohlrabi leaves (18.2 ± 1.3) as compared to fresh cabbage leaves (14.8 ± 1.1). NDF content in cabbage and kohlrabi were found low however fresh kohlrabi leaves (19.3 ± 1.1 %) had comparatively higher than fresh cabbage leaves (17.9 ± 0.9 %). However, fresh cabbage leaves (14.1 ± 1.3 %) had higher ADF content as compared to fresh kohlrabi leaves (10.9 ± 1.2 %). Crude ash content were observed higher in kohlrabi (18.7 ± 0.3 %) than cabbage leaves (20.5 ± 0.6 %).

Table 4.1. Chemical composition of fresh cabbage and kohlrabi leaves (%) DM basis

Item	Fresh cabbage leaves	Fresh kohlrabi leaves
DM	11.42 ± 1.3	16.7 ± 0.9
OM	81.3 ± 0.7	79.5 ± 0.8
CP	14.8 ± 1.1	18.2 ± 1.3
NDF	17.9 ± 0.9	19.3 ± 1.1
ADF	14.1 ± 1.3	10.9 ± 1.2
Ash	18.7 ± 0.3	20.5 ± 0.6

DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin

To our knowledge, this is first ever study suggesting nutrient composition of fresh kohlrabi leaves and its silage. Nutrient composition of kohlrabi leaves was not documented prior to this study. Moreover, limited literature is reported on cabbage and kohlrabi leaves and their silages. For this reason, we have compared nutritional composition of these feedstuffs with other members of the same family that exhibit same characteristics regarding nutritional composition. The results of current study about nutritional composition are in agreement with the findings of earlier studies

(Nkosi et al., 2016; Cao et al., 2018; Mahgoub et al., 2018) reported similar values of DM, OM, CP and crude ash but higher values of NDF and ADF in cabbage and kohlrabi leaves. Moreover, previous studies (Eván et al., 2019; Eván et al, 2018; El-Shinnawy et al., 2011; Negesse et al., 2009) have reported lower concentrations of DM, crude ash, higher values of OM and similar values of NDF and ADF in fresh cabbage waste. Moreover, Song et al. (2020) reported comparatively similar values of NDF and ADF in cabbage and Chinese cabbage as observed in the current study. In terms of CP, Mahgoub et al. (2018) and El-Shinnawy et al. (2011) have reported similar values of CP (≤ 15%) in cabbage waste. However, Nkosi et al. (2016) and Cao et al. (2018) reported comparatively higher CP values (≤ 19%) than cabbage leaves but similar to kohlrabi leaves. However, Cha et al., (2013) found comparatively lower CP 16.63% in kohlrabi pulp and peel. The higher crude ash content were found in the present study might be due to collection of fresh cabbage and kohlrabi leaves from agricultural land on large scale. Moreover, contamination can also possible when wet leaves were spread on concrete floor for wilting process. Furthermore, silages were also placed on floor in silos and pressed with tractor tyres. All these things can be the reasons to increase the crude ash content in fresh cabbage and kohlrabi and their silages. The difference observed in chemical composition of cabbage and kohlrabi leaves compared to previous literature might be due to differences in region, season, species, variety, and stage of growth.

4.2 Chemical Composition of Cabbage and Kohlrabi Silages

Nutrient composition (% of DM) of cabbage and cabbage + kohlrabi leaves silages is presented in table 4.2. DM content of cabbage leaves silage (17.7 ± 0.2 %) were found lower than cabbage + kohlrabi leaves silage (29.0 ± 0.6 %). Likewise, cabbage + kohlrabi leaves silage (76.6 ± 1.2 %) had higher OM content than cabbage leaves silage (74.3 ± 0.8 %). Similar to parent forages, their silages showed almost similar amount of CP. Silage of cabbage+ kohlrabi leaves (18.8 ± 0.9 %) showed higher CP content than cabbage leaves silage (16.4 ± 0.7 %). Cabbage leaves silage showed higher NDF and ADF (29.2 ± 1.4 and 16.3 ± 0.8 %) content as compared to other silage (20.9 ± 1.3 and 14.7 ± 1.1 %). Cabbage silage (25.7 ± 0.7 %) had greater crude ash than cabbage + kohlrabi silage (23.4 ± 0.9 %). The pH values of cabbage leaves silage and cabbage + kohlrabi leaves silage were 5.5 ± 0.3 and 5.7 ± 0.2, respectively.

Table 4.2. Nutrient composition of Cabbage and Cabbage + kohlrabi silages (%) of DM

Parameters	Cabbage leaves silage	Cabbage + kohlrabi leaves silage
DM	17.7 ± 0.2	29.0 ± 0.6
OM	74.3 ± 0.8	76.6 ± 1.2
CP	16.4 ± 0.7	18.8 ± 0.9
NDF	29.2 ± 1.4	20.9 ± 1.3
ADF	16.3 ± 0.8	14.7 ± 1.1
Ash	25.7 ± 0.7	23.4 ± 0.9
pH	5.5 ± 0.3	5.7 ± 0.2

DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin

The results of the current study about DM, OM, and crude ash of both silages are in agreement with the findings reported by Partovi et al. (2020) and Bakshi et al. (2007) in cabbage, broccoli and cauliflower silages. Moreover, Vipond et al. (1998) examined similar values of DM, OM and crude ash in kale silage as observed in the present study. However, Meneses et al.(2020) reported similar concentrations of DM, higher values of OM, and lower content of crude ash in silages of brassica plants. In opposite to our findings, El-Shinnawy et al. (2011) reported higher DM and lower ash content in cabbage waste silage. In terms of CP content, values of both silages are in line with the findings reported by Bakshi et al. (2007) and El-Shinnawy et al. (2011). They reported 20.4% and 13.11% CP in cauliflower silage and cabbage waste silage, respectively. Moreover, Vipond et al. (1998) observed 20.1% CP in kale silage as observed in cabbage + kohlrabi leaves silage of current study. However, Meneses et al. (2020) reported comparatively higher (34%) CP in broccoli silage as compared to current study. In case of NDF results, values of both silages are in agreement with the findings reported by Bakshi et al. (2007) and Meneses et al. (2020). Moreover, Cao et al. (2011) reported similar NDF content in white cabbage silage as observed in the current study. In contrast to our finding, values of NDF of both silages were found comparatively lower previously reported by El-Shinnawy et al. (2011) and Partovi et al., (2020). ADF results of the current study are in line with the findings of Meneses et al. (2020) and Cao et al. (2011). Moorby et al. (2002) reported relatively close values of ADF in kale silage. In opposite to current findings, El-Shinnawy et al. (2011) and Partovi et al., (2020) observed comparatively lower ADF values in cabbage waste silage and broccoli silage. In terms of silage pH values, Meneses et al. (2020) observed similar pH values of broccoli silage as reported in cabbage and kohlrabi silages of the current study. However, El-Shinnawy et al. (2011) observed comparatively lower values of pH in cabbage waste silage. Very few contrast studies were reported related to nutritional composition of silages of current study. The variation in earlier and present study might be attributed to difference in region, climate, season and laboratory conditions. In comparison to commonly used silages such as corn and sorghum silages, cabbage, and kohlrabi silages had relatively similar DM content but lower OM values. The silages of the current study had lower NDF but similar ADF values as compared to sorghum and corn silages (Cattani et al., 2017). Similar DM, OM and fibrous content of brassica vegetable silages indicate that they can be used as replacement of corn and sorghum silage for ruminant feeding.

4.3 *In vivo* Apparent Nutrient Digestibility of Experimental Diets

For *in vivo* apparent digestibility trials, R1 (100% AAH), R2 (50% AHH and 50% cabbage leaves silage), and R3 (50% AHH, 25% cabbage leaves silage, and 25% kohlrabi leaves silage) rations were used. *In vivo* apparent nutrient digestibility (%) values of different experimental rations are presented in table 4.3. The results showed that significantly higher ($P<0.05$) DM digestibility was observed in R2 (67.5 ± 0.3 %) followed by R3 (66.3 ± 0.2 %) and R1 (57.9 ± 0.2 %). Similarly, OM digestibility was examined significantly higher ($P<0.05$) in R2 (69.3 ± 0.2 %) and R3 (69.0 ± 0.1 %) as compared to R1 (60.1 ± 0.2 %). DM digestibility coefficients of R1, R2, and R3 were found significantly ($P<0.05$) different from each other. However, OM digestibility of R1 and R2 were observed non-significantly ($P>0.05$) different (69.3 ± 0.2 % vs. 69.0 ± 01 %) from each other while significantly ($P<0.05$) different from R1. R2 and R3 were found non-significant ($P>0.05$) in terms of crude protein digestibility while significantly different ($P<0.05$) than R1. Crude protein digestibility was observed higher in R2 (81.5 ± 0.7 %) followed by R3 (81.4 ± 0.4 %) and R1 (78.1 ± 0.9 %).Apparent digestibility of NDF was observed significantly ($P<0.05$) higher in R2 (56.3 ± 0.1 %) as compared to R3 (52.5 ± 0.1 %) and R1 (41.1 ± 0.6 %). Similarly, digestibility of ADF was examined significantly ($P<0.05$) higher in R2 (51.4 ± 0.7 %) followed by R3 (48.9 ± 0.2 %) and R1 (39.2 ± 0.5 %). The scarcity of knowledge is present regarding *in vivo* digestibility values of cabbage and kohlrabi leaves silages in ruminants. For this reason, we have compared *in vivo* apparent digestibility values of experimental rations with the members of same family like Chinese cabbage, cauliflower, broccoli, and kale. These vegetables possess same nutrient and chemical characteristics as cabbage and kohlrabi.

Table 4.3. *In vivo* apparent nutrient digestibility (%) in rams (n = 9) fed different experimental rations

Parameters	R1	R2	R3
DM	57.9 ± 0.2^{c}	67.5 ± 0.3^{a}	66.3 ± 0.2^{b}
OM	60.1 ± 0.2^{b}	69.3 ± 0.2^{a}	69.0 ± 0.1^{a}
CP	78.1 ± 0.9^{b}	81.5 ± 0.7^{a}	81.4 ± 0.4^{a}
NDF	41.1 ± 0.6^{c}	56.3 ± 0.1^{a}	52.5 ± 0.1^{b}
ADF	39.2 ± 0.5^{c}	51.4 ± 0.7^{a}	48.9 ± 0.2^{b}

$^{a\text{-}c}$ Values with different superscripts letters in a row differed significantly (P<0.05, n = 9), SEM = standard error of mean, R1= hay (100), R2= hay and cabbage silage (50:50), R3= hay and cabbage + kohlrabi silage (50:25:25), DM = dry matter, OM = organic matter, CP = crude protein, NDF = acid detergent fiber, ADF = acid detergent fiber

The results of DM and OM digestibility are in consent with an earlier study that showed 70.15% and 68.15% of DM and OM digestibility of cabbage waste silage offered with concentrate feed mixture (El-Shinnawy et al., 2011). Similarly, DM and OM apparent digestibility was observed ≤70% when 20% broccoli silage was served with concentrates to fattening lambs (Partovi et al., 2020). However, Nkosi et al. (2016) reported comparatively lower 64% and 65% of DM and OM digestibility of cabbage leaves served 20% with basal diet to sheep. Cabbage and kohlrabi leaves are extensively fermented in the rumen due to less fibrous content and high moisture level. In contrast to current study, Wadhwa et al. (2006) examined 82.1% and 88.7% DM and OM digestibility in goat fed with fresh cabbage leaves. Moreover, Meneses et al. (2020) offered a ration containing broccoli by-product silage and 45% alfalfa hay and observed comparatively higher DM digestibility (> 80%) compared to the results of the current study. In terms of CP digestibility, our results are in agreement with the finding of Meneses et al. (2020) and Wadhwa et al. (2006) who observed 83% and 84.9% CP digestibility in broccoli and cauliflower silage offered with alfalfa hay. Contrary to current findings, CP digestibility values of current study were comparatively higher than the values reported by El-Shinnawy et al. (2011) who examined 71.9% CP digestibility in cabbage waste silage offered with concentrate feed mixture. The protein in silage usually has high rumen degradability. Silage based feeding provide highly degradable protein digestibility in rumen due to proteolysis during ensiling process (Herremans et al., 2019). Wadhwa et al. (2006) explained that cabbage vegetable has high soluble sugars and water soluble protein fractions. The high DM and protein digestibility values of current study are also due to soluble sugar and protein which are more subjected to digestible in rumen. In case of NDF digestibility, findings of the present study are in quite consent with values previously reported by Moorby et al.

(2002) who observed 49% of NDF digestibility of kale and barley mix silage. Moreover, Nkosi et al. (2016) observed relatively close values of NDF digestibility in cabbage as seen in present study. However, higher digestibility of NDF in cabbage waste silage was examined by El-Shinnawy et al. (2011) as compared to the current study. The current study observed relatively lower NDF digestibility than the values reported by Partovi et al. (2020) in broccoli silage. The results of ADF digestibility are in range with the findings reported by Moorby et al. (2002) in kale silage. However, Menses et al. (2020) reported comparatively higher ADF digestibility in broccoli silage than the present study. Similarly, ADF digestibility values of brassica plants observed in the present study were relatively lower than reported by Partovi et al. (2020).The *in vivo* apparent digestibility trials showed no adverse effects on FI, growth and performance of animals. Moreover, animals maintained the body weight as they had before starting of the research trials. The variation in digestibility values of cabbage and kohlrabi in current study was observed due to addition of alfalfa hay in animal diet from those studies who offered brassica vegetable leaves alone to animal. Brassica vegetables are rapid degradable in rumen and digestive tract of animals. Alfalfa hay was added to animal feeding to minimize the metabolic disorder in sheep due to high moisture and low fibrous content of cabbage and kohlrabi silages.

Test feed apparent digestibility of nutrients from the mixed diet is illustrated in table 4.4. Individual DM, OM, CP, NDF and ADF digestibilities of alfalfa hay, cabbage leaves silage and cabbage + kohlrabi leaves silage, respectively were calculated using difference technique equation. The results showed that cabbage silage (77.1 and 78.4 %) had higher DM and OM digestibility as compared to cabbage + kohlrabi leaves silage (74.8 and 78.0 %) and alfalfa hay (57.9 and 60.1 %). Likewise, CP, NDF and ADF digestibility values were observed higher in cabbage leaves silage (85.1, 71.4 and 66.2 %) followed by cabbage + kohlrabi leaves silage (84.7, 63.8 and 58.1 %) and alfalfa hay (78.1, 41.1 and 39.2 %).Apparent digestibility values of DM, OM, CP, NDF and ADF of alfalfa hay are in agreement with the finding reported by Doran et al. (2007) and Wildeus et al. (2007). They offered alfalfa hay to sheep to check the total tract digestibility. Moreover, individual digestibility coefficients of cabbage leaves silage and cabbage + kohlrabi leave silage are in the range with values documented by Meneses et al. (2020), Wadhwa et al. (2006) and Partovi et al. (2020).

Table 4.4. Test feed digestibility (%) from the mixed diet using difference technique equation

Parameters	Alfalfa hay	Cabbage leaves silage	Cabbage + kohlrabi leaves silage
DM	57.9	77.1	74.8
OM	60.1	78.4	78.0
CP	78.1	85.1	84.7
NDF	41.1	71.4	63.8
ADF	39.2	66.2	58.1

DM = dry matter, OM = organic matter, CP = crude protein, NDF = acid detergent fiber, ADF = acid detergent fiber

All the digestibility values of current study indicated that cabbage and cabbage + kohlrabi silages had higher digestibility in ruminants as compared to alfalfa hay. Furthermore, in comparison to commonly used silages such as corn and sorghum silage, cabbage and cabbage + kohlrabi leaves silages showed higher nutrient digestibility coefficients. Oliveira et al., (2019) reported <70% DM, CP, and NDF digestibility of corn silage when it was included in sheep diet. Similarly, Almeida et al., (2019) observed <73% DM and OM digestibility and <55% NDF digestibility of sorghum silage when fed to sheep. Later validation of the above mentioned study was done by Reis et al., (2020) who observed <73% DM and OM apparent digestibility in lambs fed with corn silage. All these studies demonstrate that cabbage and cabbage + kohlrabi leaves silages have relatively equal or higher nutrient digestibility than alfalfa hay, corn silage, and sorghum silage, which suggests that these feedstuffs could be used as alternatives of conventional feed sources for efficient ruminant production in poorly resourced areas. Moreover, previous studies on the dietary inclusion of brassica vegetables in the animal diet showed body weight, milk fat, and growth performance parameters of animals were increased (Nkosi et al., 2016; Yi et al., 2015; Ngu and Ledin, 2005). It showed that brassica vegetables can be included in the diet of ruminants at an appropriate level to replace conventional feedstuffs without posing any adverse effect.

4.4 *In vitro* True Digestibility of feedstuffs using Ankom Daisy^{II} Incubator

The present experiment was conducted to determine *in vitro* true digestibility of cabbage leaves silage, cabbage + kohlrabi leaves silage, fresh cabbage leaves, and fresh kohlrabi leaves using Ankom Daisy^{II} Incubator. *In vitro* true digestibility, NDF and OM digestibility values are presented in table 4.5 and figure 4.1. All the values of IVTD, NDF and OM digestibility of fresh cabbage leaves, fresh kohlrabi leaves, cabbage leaves silage and cabbage + kohlrabi leaves silage were found non-significant (P>0.05). All the feedstuffs had high digestibility (%) similar to the other forages incubated for 48 hours. The results showed that higher *in vitro* true digestibility (DM basis) was observed in fresh cabbage leaves (93.5 ± 0.9 %) followed by cabbage + kohlrabi leaves silage (92.1 ± 0.9 %), cabbage leaves silage (91.4 ± 1.3 %), and fresh kohlrabi leaves (90.9 ± 1.4 %). Similarly, fresh cabbage leaves (93.9 ± 0.9 %) had higher *in vitro* true digestibility (as fed basis) as compared to cabbage silage (92.1 ± 1.2 %), cabbage + kohlrabi leaves silage (92.6 ± 0.9 %), and fresh kohlrabi leaves (91.4 ± 1.3 %). Likewise, *in vitro* NDF digestibility was observed higher in fresh cabbage leaves (64.1 ± 1.2 %), followed by fresh kohlrabi leaves (63.6 ± 1.1 %), cabbage leaves silage (62.7 ± 1.6 %) and cabbage + kohlrabi leaves silage (62.5 ± 1.2 %). Moreover, higher *in vitro* OM digestibility was observed in fresh cabbage leaves (68.7 ± 1.4 %) followed by cabbage + kohlrabi leaves silage (68.4 ± 1.1 5), fresh kohlrabi leaves (67.9 ± 0.9 %), and cabbage leaves silage (65.2 ± 0.8 %).

Table 4.5. *In vitro* true digestibility (dry mater and as fed based), NDF and OM digestibility of different feedstuffs

Parameters	Cabbage leaves silage	Cabbage + kohlrabi leaves silage	Fresh cabbage leaves	Fresh kohlrabi leaves
IVTD (DM)	91.4 ± 1.3	92.1 ± 0.9	93.5 ± 0.9	90.9 ± 1.4
IVTD (AF)	92.1 ± 1.2	92.6 ± 0.9	93.9 ± 0.9	91.4 ± 1.3
NDFD	62.7 ± 1.6	63.6 ± 1.2	64.1 ± 1.2	63.2 ± 1.1
OMD	65.2 ± 0.8	68.4 ± 1.1	68.7 ± 1.4	67.9 ± 0.9

IVTD (AF) = *in vitro* true digestibility as fed bases, IVTD (DM) = *in vitro* true digestibility as dry matter bases, NDFD = neutral detergent fiber digestibility, OMD = organic matter digestibility

The characteristics such as higher moisture, lower fibrous content (NDF, ADF and lignin), high water soluble sugar and protein make cabbage and kohlrabi more and rapidly degradable in the rumen. The results of the current study are in consent with the findings of Eván et al. (2019) who found comparatively higher in situ DM digestibility (> 86%) of white cabbage, savoy cabbage, and red cabbage. Similarly, values of this study are within the corresponding range reported by Song et al. (2020) examined >90% in situ DM digestibility of cabbage and Chinese cabbage by-products incubated for 48 hours suggesting that brassica vegetables are rapidly degradable in the rumen due to higher soluble sugars and protein content.

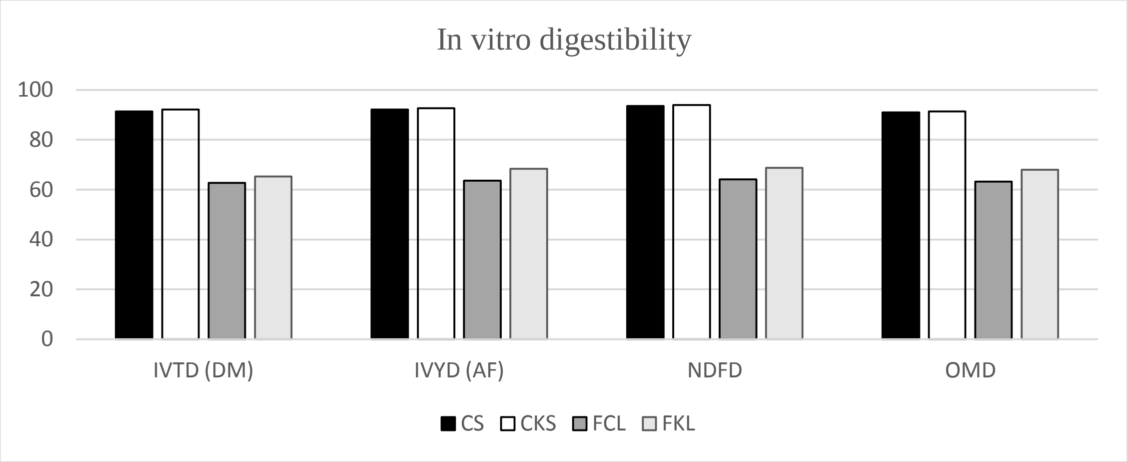

Figure 4.1. *In vitro* true digestibility, *in vitro* NDF and OM digestibility of various feedstuffs using Ankom Daisy^{II} Incubator at 48 hours incubation

However, the current study observed comparatively higher *in vitro* true digestibility of cabbage leaves than reported by Negesse et al. (2009) and Mahgoub et al. (2018). Mekasha et al., (2002) observed comparatively lower *in vitro* DM digestibility (80.4%) of cabbage waste than the present study. Brassica vegetables and their silages have a good nutrient profile and digestibilities and compare well with conventional feedstuffs such as alfalfa hay and corn silage. The results obtained agreed with those reported by Meneses et al. (2020) who observed 95.6% *in vitro* DM digestibility of broccoli silage after 48 hours of incubation. However, Cao et al. (2011) examined 50.3%, 41.6%, and 52.3% *in vitro* DM digestibility of white cabbage, Chinese cabbage, and red cabbage silages incubated for 6 hours. Azevêdo et al., (2012) reported similar *in vitro* NDF digestibility than the present study. They reported 69.5% and 70% *in vitro* NDF digestibility of wild cabbage residues and wild cabbage talk, respectively. Moreover, *in vitro* NDF digestibility values of the current study are relatively close previously reported by Wadhwa and Bakhsi (2005) who examined 76.3% *in vitro* NDF digestibility of cabbage leaves determined through *in vitro* gas production technique. The good *in vitro* NDF digestibility of cabbage and kohlrabi in current study could be attributed to lower lignin content of the feedstuffs. The presence of lignin tends to increase the indigestible ratio of fibers in feed. In case of OM digestibility, Negesse et al. (2009) documented OM digestibility of cabbage leftover (75.8%) relatively close to the values observed in the current study. Moreover, Marino et al. (2010) reported relatively close values of OM digestibility (78.3%) of cauliflower leaves in comparison to current findings. However, their results of OM digestibility in cabbage and broccoli leaves were comparatively higher than present study. The higher DM, OM and NDF digestibility values suggest that cabbage and kohlrabi are extensively fermented in the rumen. Moreover, *in vivo* digestibility trials indicated that brassica vegetables had no adverse effects on FI, growth and performance of animals. It is concluded that they can be used as complete feed for ruminants.

4.5 Total Phenolic Content in Cabbage and Kohlrabi Leaves and their Silages

TPC in the fresh leaves and silages of cabbage and kohlrabi are presented in table 4.6 and figure 4.2. The results revealed that significantly higher ($P<0.05$) TPC were observed in fresh kohlrabi leaves (455.5 ± 0.3) and fresh cabbage + kohlrabi leaves (443.5 ± 0.1) as compared to the others. The TPC in cabbage leaves silage (236.4 ± 0.1) did not indicate any significant change ($P>0.05$) than cabbage + kohlrabi leaves silage (227.4 ± 0.4). However, cabbage leaves silage had significantly higher TPC as compared to the fresh cabbage leaves (236.4 ± 0.1 vs. 213.1 ± 0.3; respectively), which indicates that the ensiling process can alter the TPC in feedstuffs.

Table 4.6. TPC (mg GAE 100 g-^{1}DW) of fresh cabbage and kohlrabi leaves and their silage

Groups	TPC
Fresh cabbage leaves	213.1 ± 0.3^{c}
Fresh kohlrabi leaves	455.5 ± 0.3^{a}
Fresh cabbage + kohlrabi leaves	443.5 ± 0.1^{a}
Cabbage leaves silage	236.4 ± 0.1^{b}
Cabbage + kohlrabi leaves silage	227.4 ± 0.4^{bc}

$^{a-c}$ Values with different superscripts letters in a column differed significantly ($P<0.05$, n = 12), TPC = total phenolic content

These results were in accordance with an earlier study that showed 205.7 mg GAE/100 g DW TPC in white cabbage (Karoui et al., 2018). However, TPC in cabbage leaves were not similar to that reported by Seong et al. (2016). Similarly, Liang et al. (2019) reported TPC in different varieties of cabbage ranged from 86.64 to 153.94 mg GAE 100 g-^{1} DW. Moreover, Isabelle et al. (2010) observed 186 mg GAE 100 g-^{1} DW TPC in red cabbage. TPC in kohlrabi observed in the current study were in agreement reported by Ranyi et al. (2017). They reported 411.03 mg GAE 100 g-^{1} DW TPC in kohlrabi. Whereas, the current study showed relatively higher TPC in fresh kohlrabi and kohlrabi silage than previously reported by Yagar et al. (2016). They found TPC in kohlrabi ranged from 164.7 to 275.8 mg GAE 100 g-^{1} DW. Furthermore, TPC in kohlrabi were not similar to the values reported by Kim et al., (2014) and Patras, (2020). The difference in current and previous studies might be due to topography, season, plant varieties, and extraction methods.

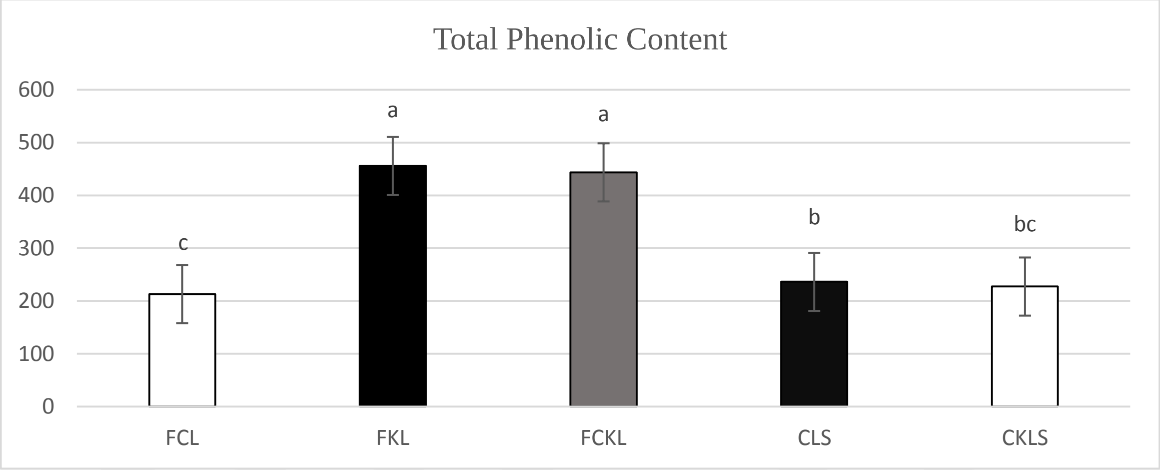

Figure 4.2. Total phenolic contents (mg GAE/100 g-^{1} DW) of fresh cabbage and kohlrabi leaves and their silage ($P<0.05$)

4.6 Antioxidant Activity of Fresh Cabbage and Kohlrabi Leaves

Antioxidant activity (µM Trolox equivalent g^{-1}) of fresh cabbage and kohlrabi leaves was determined through TAEC assay. The TAEC assay is based on the scavenging of the ABTS radical anions method. The antioxidant activity of fresh cabbage and kohlrabi leaves are presented in Table 4.7 and figure 4.3. Significantly higher ($P<0.05$) antioxidant activity was observed in kohlrabi leaves (664.6 ± 0.1) followed by fresh cabbage + kohlrabi (405.6 ± 0.1) and cabbage leaves (149.4 ± 0.1).

Table 4.7. Antioxidant activity (µM Trolox equivalent g^{-1}) of fresh cabbage and kohlrabi leaves

Item	Antioxidant activity
Fresh cabbage leaves	149.4 ± 0.1^{c}
Fresh kohlrabi leaves	664.6 ± 0.1^{b}
Fresh cabbage + kohlrabi leaves	405.6 ± 0.1^{a}

$^{a\text{-}c}$ Values with different superscripts letters in a column differed significantly ($P<0.05$)

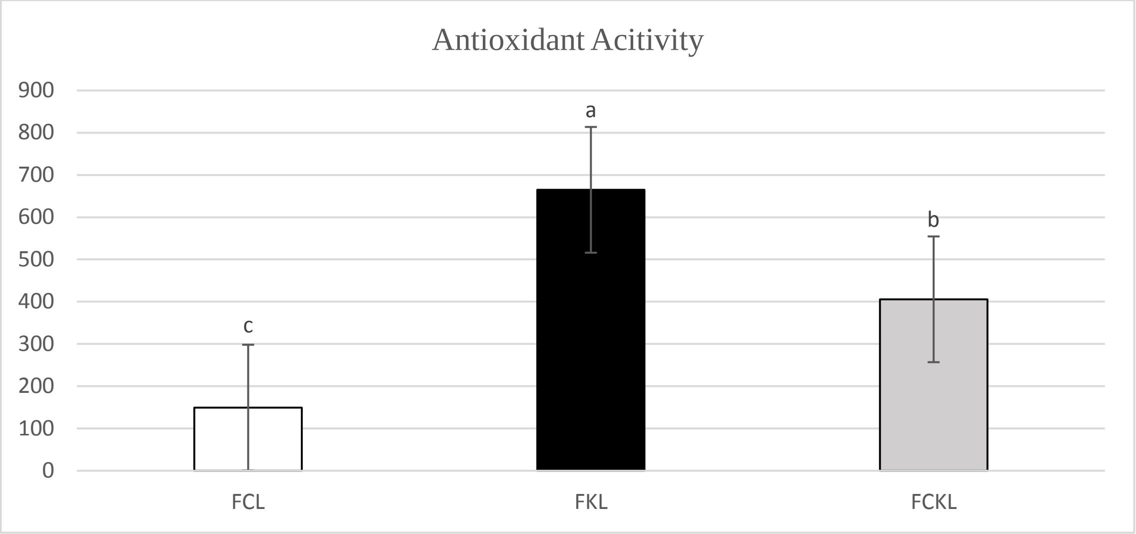

Figure 4.3. Antioxidant activity (µM Trolox equivalent g^{-1}) of fresh cabbage and kohlrabi leaves ($P<0.05$)

The results of the current study are in line with the range reported by Seong et al. (2016) and Kim et al. (2014). They reported antioxidant activities 175.2 µM Trolox equivalent g^{-1} in cabbage (Seong et al., 2016) and 664.3 µM Trolox equivalent g^{-1} in kohlrabi (Kim et al., 2014). The antioxidant activity in cabbage was relatively higher than the values observed by Managa et al. (2020). The values of antioxidant activity in cabbage were reported comparatively lower as compared to the current study (Liang et al., 2019; Peñas et al., 2011). Whereas, antioxidant activity values observed in kohlrabi were not similar previously reported by Vicas et al., (2013). The difference in current and previous studies might be due to topography, season, plant varieties, and extraction methods.

CHAPTER V

CONCLUSION

The livestock sector is a major contributor in raising the rural economy and supports the livelihoods of people providing a range of many important benefits. Human population growth will be more than 10 billion in 2050 and demand for animal products is increasing to a greater extent and will continue to increase in the near future. Correspondingly, it encourages the livestock population by 70%. To accomplish the targeted level of production, efficient livestock feeding is very important as feed is a major determinant of livestock production.

Livestock production is restrained around the world due to an inadequate supply of feed. Urbanization and industrialization are continuously reducing farmable land resulting in low animal productivity. The transformation of intensive livestock production towards grains feeding, the high price of conventional feedstuffs, and the depletion of agricultural land encourage us to find alternative sources to increase animal productivity in poor resource areas. The use of local feed resources, crop waste, and residues as livestock feed is a necessary precondition for commercial production. Crop residues are produced in a significant quantity that can potentially be used as alternatives of conventional feedstuff for ruminant and economize the feeding cost. This strategy would increase the economic benefits for farmers and contribute to alleviating the environmental problems associated with their elimination.

Fresh cabbage and kohlrabi leaves and their silages were used to evaluate their chemical composition, *in vivo* apparent digestibility, *in vitro* true digestibility, NDF, and OM digestibility, total phenolic contents, and antioxidant capacity. Cabbage and kohlrabi leaves and their silages had low DM, high CP, and low fibrous content. Silages had good physical characteristics, vinegary smell with a pH of 5.4 to 5.7. Test feeds (cabbage leaves silage and cabbage + kohlrabi leaves silage) showed significantly higher ($P<0.05$) *in vivo* apparent digestibility of DM, OM, CP, NDF, and ADF than alfalfa hay. DM had a high *in vitro* degradability and *in vivo* digestibility. *In vitro* true digestibility was greater than 90% in all feedstuffs at 48 hours incubation indicated that cabbage and kohlrabi are rapidly and extensively degradable in the rumen due to lower fibrous content and higher moisture and soluble sugars. The higher total phenolic content and antioxidant activity of cabbage and kohlrabi leaves showed that they could be added in animal feed to provide bioactive components that can alter the rumen fermentation in beneficial ways. Moreover, the antioxidant activity of phytochemicals can reduce disease occurrence, stress, and lipid oxidation.

The chemical composition, nutrient digestibility, and phenolic contents of cabbage and kohlrabi leaves suggest that they could be excellent non-conventional feed sources for ruminants, equivalent to any conventional feedstuffs such as alfalfa hay, corn silage, and sorghum silage. Dietary inclusion of cabbage and kohlrabi leaves up to 50% for sheep is equivalent to alfalfa hay in terms of feed consumption, feed efficiency, and nutrient digestibility. These feedstuffs had no adverse effect on animal health status, growth, and performance. Moreover, these feed resources may help in economizing production cost and providing green fodder during feed shortage periods.

Further investigations on the viability of using fresh cabbage and kohlrabi leaves and their silages are required for long term feeding trials with ruminants on their performance. Moreover, much research is needed to conclude to what extent the anti-nutritional factors (Glucosinolates and S-methyl-L-cysteine sulphoxide) in cabbage and kohlrabi diets could cause depression in growing ruminants. Limited results of the current study warrant further research on dairy and beef cattle to investigate the milk production and fattening potential of cabbage and kohlrabi leaves and silages. More studies are required to improve the nutrient profile of wastes obtained from leafy vegetables and to discard the potential negative effects of anti-nutritional factors associated with cabbage and kohlrabi on growth performance parameters of the animal. Moreover, research-based information should be provided to farmers to raise awareness in rural areas about their utilization, preservation, and elimination methods. Crop residues can be preserved for future use in the form of hay, silage, and haylage. Their nutrient profile can also be increase through the incorporation of urea, ammonia, and enzymes. Further research is recommended in a promising way at field conditions to preserve high moisture crop residues. These feedstuffs are more vulnerable to spoilage due to their high waste content.

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CURRICULUM VITAE

I am Muhammad Zeeshan Akram, born on September 21, 1994 in Gujranwala, Pakistan. I have acquired a bachelor degree (Doctor of Veterinary Medicine, DVM) with distinction from the University of Veterinary and Animal Sciences (UVAS) Pakistan. I stood ranked second among all campuses of the university and awarded a silver medal at graduation level. I was selected for the Mevlana Exchange Program in Turkey (2017-2018) during bachelor degree at Niğde Omer Halisdemir University and worked on the evaluation of different feedstuffs for maximizing animal production. Moreover, Erasmus Plus Program (2019-2020) helped me to learn, explore, and grow a little more. During Erasmus, I completed two traineeships at Poznan University of Life Sciences Poland and UTP University of Science and Technology Poland in ruminant nutrition and ovine molecular genetics, respectively. My MPhil research is related to ruminant nutrition in which I have worked on “*Effect of cabbage and kohlrabi leaf silages on in vitro and classical nutrient digestibility in Akkaraman rams as an alternative feed source”.* Akkaraman rams and Ankom^{II} Daisy Incubator were used for in-vivo and in-vitro digestibility trials respectively. *In vivo* apparent digestibility coefficients, *in vitro* true digestibility, *in vitro* NDF digestibility, *in vitro* OM digestibility, total phenolic contents, and antioxidant capacity of feedstuffs were evaluated. I have also worked along with other colleagues on monogastric animals in different projects like the effects of garlic powder on growth and carcass characteristics of quails.