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MASTER THESIS

NİĞDE ÖMER HALISDEMIR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND PPLIED SCIENCES

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

IN VITRO DIGESTIBILITY OF ALFALFA SILAGE SUPPLEMENTED WITH DIFFERENT AMOUNT OF ORANGE AND MANDARIN PULP

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Supervisor
Assistant Prof. Dr. Sema YAMAN FIRINCIOĞLU

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The study titled **"*In vitro* digestibility of alfalfa silage supplemented with different amount of orange and mandarin pulp"** and presented by **Maira TARIQ** under the supervision of **Dr. Sema YAMAN FIRINCIOĞLU**, has been recognized 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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CONFIRMATION:

This thesis has been found appropriate at the date of 09/09/2021 by the jury mentioned above who have been designated by Board of Directors of Graduate School of Natural and Applied Sciences and has been confirmed with the resolution of Board of Directors dated …./…./2021 and numbered ……

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DECLARATION

It is certified that I have written this thesis by myself. I further confirm that all information included in this thesis is scientific and is in accordance with the university rules and regulations. Any materials that I have used from external sources as well as help received and all sources used in finalizing this research work and preparing this thesis, all have been acknowledged in the thesis.

Maira TARIQ

SUMMARY

IN VITRO DIGESTIBILITY OF ALFALFA SILAGE SUPPLEMENTED WITH DIFFERENT AMOUNT OF ORANGE AND MANDARIN PULP

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Citrus fruits consumption and dispensation produce a momentous quantity of by-products as left-over, which can be used as potential feed for animals. This study was performed to investigate the *in vitro* digestibility of alfalfa silage supplemented with different amount of orange and mandarin pulp. Orange and mandarin pomace were added in a ratio of 0.20, 0.25 and 0,30 to alfalfa harvested at beginning of flowering stage, wilted for 24 hours and were ensiled for 60 days, respectively. Dry matter (DM%), ash (XA%), crude protein (CP%), neutral detergent fiber (NDF%), and acid detergent fiber (ADF%) were determined for alfalfa hay, orange, mandarin, and their silages. DM and CP content were recorded highest for alfalfa silage, NDF was recorded highest for ALFM 25 silage and ALFO30 silage had the highest XA content and lowest ADF content. The orange and mandarin supplementation improved the *in vitro* true digestibility, DM and NDF digestibility. The *in vitro* digestibility of DM (83.38±0.97) and NDF (57.08±2.51) was highest in ALFO 30, while the ALF (control) had the lowest values for IVTD (DM) (75.24±1.15) and NDFD% (33.22±3.12). It was concluded that ensiling alfalfa with orange (30%) pulp will increase the chemical composition of silage and enhances the *in vitro* true digestibility.

Keywords: orange, mandarin, pulp, alfalfa silage, *in vitro* digestibility

ÖZET

FARKLI MİKTARDA PORTAKAL VE MANDARİN KURUMU İLE TAKVİYE EDİLEN YONCA SİLAJININ *IN VITRO* SİNDİRİLEBİLİRLİĞİ

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Narenciye tüketimi ve dağıtımı, hayvanlar için potansiyel yem olarak kullanılabilecek, çok önemli miktarda kalıntı olarak yan ürün üretir. Bu çalışma, farklı oranlarda portakal ve mandalina posası ilave edilmiş yonca silajlarının *in vitro* sindirilebilirliğini araştırmak amacıyla yapılmıştır. Portakal ve mandarin posası, çiçeklenme başlangıcında hasat edilmiş ve 24 saat soldurulmuş yoncaya %20, %25 ve %30 oranında eklenerek 60 gün boyunca silaj olarak muhafaza edilmiştir. Ham besin maddeleri açısından analiz edilen örnekler arasında istatiksel olarak önemli bir fark bulunmamıştır. Ancak, havada kuru madde (HKM) ve HP içerikleri sayısal olarak en yüksek yonca silajında, en yüksek NDF değeri ALFM25 silajında bulunmuş iken, en yüksek HK ve en düşük ADF ALFO30 silajında bulunmuştur. Portakal posası ilavesi yonca silajlarında *in vitro* gerçek sindirilebilirliği KM ve NDF sindirilebilirliğini artırmıştır ($P<0.05$). Mandarin posası ilavesi yonca silajlarında *in vitro* sindirilebilirlik üzerinde istatiksel olarak önemli etki oluşturmamıştır. *In vitro* KM (83.38±0.97) ve NDF sindirilebilirliği (57.08±2.51) en yüksek ALFO30 silajında bulunurken, ALF (control) silajı, IVTD (KM) (75.24±1.15) and NDFDS% (33.22±3.12) bakımından en düşük bulunmuştur. Yoncanın portakal posası (%30) ile silajlanmasının, *in vitro* gerçek sindirilebilirliğini artıacağı sonucuna varılmıştır.

Anahtar Sözcükler: portakal, mandarin, posa, yonca silajı, *in vitro* sindirilebilirlik

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SYMBOLS AND ABBREVIATIONS

Symbols	Abbreviations
%	Percentage
°C	Degrees Celsius
g	Grams
Kg	Kilograms
L	Liter
Mg	Milligrams
mL	Milliliters

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
OS	Orange Silage
MS	Mandarin Silage
IVTD	In Vitro True Digestibility
ALF S	Alfalfa Silage

CHAPTER I

INTRODUCTION

The global significance of ruminants and their product supplies are increasing as the consumer preference in low-to-middle-income countries rises due to population expansion and economic growth (Pulina et al., 2017; Wadhwa et al., 2015). In this context, about 60% to 70% more livestock goods will be required to feed a population that is predicted to grow from 7.3 billion in 2015 to 9.5 billion in 2050, with the majority of this expansion coming from low-to-middle-income countries (Pulina et al., 2017; Thornton, 2010).

Hence, there is a universal need to boost ruminant productivity in declining fertile farmland and pastureland in a sustainable manner. (Pulina et al., 2017). Genetic, diet, and raised practices all have an impact on animal production. A quality feed is a key aspect in meeting animals' growth demands in order to attain optimum production and a sustainable livestock business, resulting in increased farmer revenue (Wiyatna et al., 2012). Livestock (ruminants) can assimilate low quality feedstocks and rich in fiber to produce a qualifying ration (Lunagariya et al., 2017). Forages used to make ration must have sufficient nutritional content (carbohydrate, protein, mineral, water, fat and vitamin) for ruminants to achieve the best results. Intake for ruminants must contain certain ingredients and be prepared in a specific way. For maximum output, livestock must be fed in a balanced and cost-effective manner. The lack of food and imbalanced feeding methods are the major causes of lower livestock productivity. The need of hour is to reduce feed costs and labor while increasing productivity. This can be accomplished via blending concentrates, primarily made of locally accessible by products and fodder sections of the feed to have a complete intake, also known as a total mixed ration. Complete diet prepared from fiber crop residues like alfalfa is a great approach to raise intake, enhance food efficiency, and improve animal productivity. The whole feed system is gaining popularity since it permits for increased use of crop residues, Agri-by products, and non-conventional feeds in dairy rations, allowing for increased productivity and minor feeding costs. (Schmidt et al., 2009; Yakin et al., 2012).

Alfalfa (*Medicago sativa* L.) is one of the world's highly significant legume forages because of its excellent nutritional value, high yields and flexibility. It is a major source of protein for livestock and is a staple in the diets of beef and dairy cattle, sheep, goats, horses, and other household animals. It's grown on over 35 million hectares in over 80 countries (Radovic et al., 2009). Alfalfa is generally utilized as animal feed, even though its slender stems and sprouting seeds are occasionally ingested by humans. It's usually cultivated for hay, and it's frequently used for haylage or silage, dried to make pellets or meal, or used fresh in cut-and-carry operations or grazing (Grassland, 2011). Dehydration factories in numerous countries make its pellets with specified protein component for special markets. Alfalfa can be produced as pure grass or as a pasture when raised together with grasses or legumes. Industrially generated pigment-rich protein concentrates made by pressing the aerial component are used in chicken and pig nutrition (Cook et al., 2005). Silage is an effective conservation measure even in severe situations. Because alfalfa is low in carbohydrates, it should be augmented with carbon resources, such as pulverized grains like barley or wheat and supplemented to start fermentation (Marumo et al., 2016). Due to lower leaf losses, alfalfa silage often retains more nutrients than alfalfa dry hay. Furthermore, rainy weather damage is less likely to occur since wilting on the field requires less time to acquire the desired dry matter (DM) level. Wetter feeds may also be more digestible and palatable for cattle, particularly in hot and dry climates. Alfalfa silage is more suitable for ruminant total mixed rations (TMRs). Pre-wilted alfalfa or fresh alfalfa can be used to make alfalfa silage. To avoid nutrient leaching, the moisture of the crop before silage should be 50 to 70 percent (Mason, 1998). Due to the more water content of fresh alfalfa, it is recommended to add ground grain, dried beet pulp, wheat straw and other materials at the bottom of the silo to avoid liquid loss. If the silage is too wet, anaerobic bacteria like Clostridium can proliferate and break down the proteins. Organic acids (propionic acid, formic acid and formic acid + formaldehyde) and calcium salts are examples of additives that can assist lower pH and increase conservation (low pH is not favorable to the growth of Clostridia) (Mason, 1998; Mauries, 2003). If the raw material is excessively dry or compacted insufficiently, oxygen can become confined in the silage, causing mold and yeast to grow. To remove oxygen from the alfalfa, it must be delicately chopped (Meisser et al., 2005). Because wilting minimizes water content and protein breakdown, pre-wilting is the greatest strategy to increase forage value. However, when the moisture content is greater than 50%, leaf losses are significant, resulting in protein loss (Mauries, 2003). Alfalfa is a high-nutritive feed crop that is

frequently used in the diets of high-yielding dairy cows (Schmidt et al., 2009; Albrecht and Beauchemin, 2003). It holds a great buffering capability and is rich in highly degradable crude protein (CP) content and is low in water-soluble carbohydrates (WSC) content (Buxton and O'Kiely, 2003). Ensiling alfalfa in addition to orange molasses and pulp can improve the quality of silage, gas production, and DM digestibility *in vitro* (Besharati et al., 2017).

The technique of administering crop and food industry by-products to animal's dates to the domestication of livestock by mankind. It has two main benefits: it reduces livestock's dependency on cereals that can be consumed by people, and it eliminates a need for expensive waste control procedures. Citrus by-products have seen a substantial surge in use in recent times. (Khan et al., 2014; Ding et al., 2012). Citrus pulp is rich in antioxidants and enhances lipid profile and digestion in the rumen, all of which are linked to functional elements such as phenolic chemicals (Ghasemi et al., 2009). Citrus pulp is high in polyphenols (M'Hiri et al., 2014). Those pulps are high in flavonoids and hydroxycinnamic acids (sinapic acid and ferulic acid) (Abeysinghe et al., 2007; Nogata et al., 2006). Citrus flavonoids have been proven safe and nontoxic mutually in *vivo* and *in vitro* feeding studies (Li et al., 2013, 2014a). Citrus pulps have nutritional advantages, which would be helpful in animal diet and nutrition, save feed costs of production, and aid the planet by minimizing additional waste products. Feeding bioavailability is a key metric that may be used to estimate the amount of nutrients and food that the digestive system can digest (Mayulu et al., 2018).

In vitro rumen fermentation is a fast technique of examination which has been usually used to investigate feed breakdown, identify minute changes in fermentation properties between supplements, and enable for more regular testing. This method allows for the preference of feed or feed components that promote high microbial synthesis of proteins utilization in the rumen while maintaining high intestinal absorption. It also serves as a foundation for the growth of ruminant form of guidelines that optimize substrate modification into microbial cells (Makkar, 2015). Various groups have reported that supplementing whole feeds with various food supplements improves nutrient biodegradation. Mir et al. (2010) found that supplementing several herbs at 2% and 3% levels in total feeds for ruminants increased *in vitro* digestibility and *in vitro* organic material digestibility. Nutrient digestibility of feedstuff in ruminants are determined by

using the Ankom Daisy^{II} incubator. It is widely used as an alternate to conventional *in vitro* techniques. The system allows for the incubation of several feedstuffs in sealed polyester bags in the same incubation tank, which constantly rotates at 39.5 °C. The feed disappears from the bag during the incubation process is considered to be digestible through this procedure. The approach, which was originally designed to estimate the digestibility of feedstuffs for livestock, has been improved and modified to increase its precision and prediction accuracy (Tassone et al., 2020).

By-product that is readily available in the area feedstocks can improve digestion, as well as the microbial community can use these high-fiber by-products to fulfill their nutritional needs for health, development, breeding, and productivity. Citrus by-product is a key element of ruminant feeding in many parts of the globe, and it contains a variety of food supplements that vary depending on the source crop and system of production. The solid that left after fresh fruits have been pressed into liquid is known as citrus pulp. Citrus pulp is the dehydrated residue of grapefruit, orange and other citrus fruit pulp, peel and seedlings. Citrus pulp has a great nutritive level due to the huge quantity of easily available fructose and glucose, as well as a range of oxidative metabolism for ruminal microorganisms, such as fermentable sugars and an easily digested neutral detergent fiber (NDF) percentage.

As a result, when citrus by-product feedstuff (BPF) is replaced for starch diets, the digestible coefficient of acid detergent fiber (ADF) and NDF are improved. Gas output was boosted by adding orange pulp and molasses. The accessibility of both protein and fructose dietary fiber has a big impact on *in vitro* gas generation. (Kondo et al., 2004). *In vitro* feed organic matter digestibility and gas production were shown to be highly correlated (Menke, 1988). Because the rate of gas production can indicate digestibility in the rumen, which affects the transport rate and intake of dry matter, several scientists have effectively utilized this approach to examine the effects of feed digestibility through this relation (Negesse et al., 2009).

This study was done to determine the effects of supplementing alfalfa silage with orange and mandarin pulp on *in vitro* true digestibility in order to provide nutritionists with the necessary information to replace or at least modify traditional ruminant feed rations with citrus by products.

CHAPTER II

REVIEW OF LITERATURE

2.1 Contribution of Ruminants to Food Security for Man

The worldwide population is expected to reach 9.7 billion by 2050, with predictions for a further increase to 11 billion by 2100. (UN DESA, 2019). Not only will the world's population grow in the future decades, but per capita earnings in emerging countries are predicted to triple between 2004 and 2030 (Cirera and Masset, 2010). Families are likely to increase their meat and milk intake as income rises (Delgado, 2003). In tandem with this increase in population and income, the world's population and life expectancy are both increasing. Between now and 2050, it is expected that the population of people aged 60 and up would more than triple (UN PDI, 2001). In general, these variations in the demographic structure have made it crucial to produce more food. In fact, it is predicted that at least we must expand the food production by 70 percent (Tester and Langridge, 2010). To meet this demand, annual meat production will have to increase by more than 200 million tons. Food will be produced in a more sustainable manner in order to produce more food (FAO, 2009). Food will be produced in a more sustainable way in order to produce more food. In order to produce more food, that food will be produced in a more sustainable manner. In practice, this suggests utilizing fewer animals, less space, and less water to meet the optimum increase in milk and meat production (Gomiero et al., 2011). Domestic herbivore milk and meat account for 16 percent of the worldwide protein utilization and 8% of world's energy use. Cattle generate the majority of milk and meat from household animals, accounting for 83 percent of milk and 20 percent of meat. On the other hand, buffaloes can produce 1 percent of the world's meat and 13 percent of the world's milk. Small ruminants make a smaller contribution, providing only 5% of meat and 4% of milk. In all four species of ruminants, diverse crop-livestock production methods contribute the most to meat and milk production: 59 percent, 85 percent, and 57 percent for buffalo, cattle, and small ruminants i.e., sheep and goats, correspondingly.

The remaining output of cattle comes from grazing systems and, to a lesser extent, feedlots. Despite their fast growth ratios and large carcasses, feedlot animals are not widely used, and their total contribution to beef protein production is still small (5

percent) (FAOSTAT, 2017). Milk and meat also contribute to food and nutritional security by providing a variety of micronutrients, such as calcium, zinc, riboflavin, vitamin A, and vitamin B12, which might be hard to attain or process in enough amounts from plant-source foods alone in some areas (Murphy and Allen, 2003). This is especially true for poor demographic groups who only have accessible to bland, nutrient-deficient meals. Meat and milk, for example, are the only nutritional sources of vitamin B12 and contain easily digestible vitamin A, calcium, iron, and zinc (Randolph et al., 2007). They include the highest quantities of sulfur-containing amino acids and have the greatest concentration of threonine and lysine, which are in little amount in most plant-based meals. These nutrients are required for immune system function, rational development, and growth, among other things. As a result, nutrient-dense meals like milk and meat are especially necessary during the first 1000 days of life, as well as for nursing and pregnant women. (Allen, 2013).

2.2 Natural Feed Availability and Nutrition for Ruminant's Production

Food shortages and famine are becoming endemic in developing countries. It is associated with urbanization, industrialization, and reduction in farmable land. The necessity for animal products such as meat, milk, and eggs are 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 Role Of By-Products in Ruminants Production

By-product feedstuffs are divided into several categories, comprising stalks and residues. By-products from fruit and vegetable remains, by-products from the legume milling and cereal sector and from the oil industry are among the residues. Agricultural by-products

are high in nutrition and be able to help save animal-product expenses. The agricultural by-products would be of substantial value as a market for the primary items from which they were produced, alleviating the food crisis to some extent. Ruminants benefit from high fiber feed because their rumens contain large populations of microorganisms (Iqbal et al., 2018). However, in ruminant systems, substantially less human-edible feed is required to generate the equal quantity of animal protein products (milk, meat, or eggs) than those in monogastric systems (Mottet et al., 2017). A variety and efficient utilization of side streams, as well as increasing consumption of fibrous feeds not compatible with human or monogastric animal nutrition, might further develop the capabilities intrinsic to livestock animals within the food supply chain. Animal production techniques have changed dramatically in recent years, resulting in the containment of massive herds in limited specific intense farms, where creatures, particularly ruminants, are frequently maintained indoors. Due to the shortage of grazing on grasslands, feed costs can account for up to 70% of overall cost of production in the ruminant business. High fibrous feed such as wheat straw, rice straw etc. are frequently used in ruminants (Nayan et al., 2019).

Energy, on the other hand, is vital for animal productivity in terms of food provision. The energy utilization of compounded unconventional feedstuffs is mainly unclear. Rice straw has ME and NE quantities of 6.76 and 3.42 MJ/kg, while wheat straw has ME and NE values of 6.43 and 3.28 MJ/kg, correspondingly (Wei et al., 2018). Furthermore, straws processed with fermentation, silage, enzyme, as well as other procedures were recently shown to have increased quality before being used as ruminant fodder (Zhang et al., 2017 and Babaeinasab et al., 2015). By-products of the agricultural industry, such as grape pomace or cassava wastes, are fed to ruminants. Numerous research has found that cassava and grape by-products can help calves, dairy lambs, and cows to produce more milk. (Amaral et al., 2019; Pilajun et al., 2016). However, by-products of grape also have the ability to lessen methane production nearly 20% for Ruminants. (Moate et al., 2014). Other fruit and vegetable agro-industry by-products, such as dried grape pomace, and citrus pulp have shown a favorable association with ruminant behavior (Froetschel et al., 2014). Supplementing ruminant feeds with by-product materials is frequent, although there is no standardized additional criterion.

2.4 Alfalfa in Ruminants Diet

Forage plants, which include monocotyledonous vegetation and dicotyledonous legume with annual lifecycle or perennial lifecycle, are vital to the livestock enterprise. Alfalfa (*Medicago sativa* L.) is the broadly cultivated perennial leguminous species in several countries, accounting for the 4th biggest cultivar in terms of economic value after wheat, corn and soyabean in US (United States) (Fernandez-Cornejo et al., 2016), with a predictable global harvesting area of 30 MH (Million hectares) (Annicchiarico et al., 2015). Alfalfa is a feet high, rainy season plant that produces high-quality and high-yielding feed that sheep quickly devour and helps them acquire weight (Gelaye et al., 1990; Fisher et al., 2002). Rice straw, for example, is plentiful and inexpensive, but it is often regarded as low-quality feedstuffs. A second variety, alfalfa, is very well for high quality and is widely utilized as a supplementary feed in ruminant production around the world; however, rice straw is the most common forage resource for ruminants in tropical areas. Those two forages, which vary in the digestible of their DM in the rumen (Yang et al., 2011), share characteristics such as high plant biomass, superior nutritive value, and flexibility. Some secondary metabolites like flavonoids are found in alfalfa. Alfalfa varieties which are cultured in China was 0.6-0.9% by having 73.3% flavonoid content (Benchaar et al., 2006). Nowadays, alfalfa is frequently utilized in ruminant diet, and its flavonoid content may be responsible for some of its advantages. Moreover, it's capability to fix nitrogen via a symbiotic interaction with *Rhizobia* spp. improves soil fertility and reduces a need for fertilizer, making it a desirable crop for grazing, hay, and silage cultivation. (Radovic et al., 2009).

Ruminants are notable for their capacity to metabolize a fiber-rich forage vegetation feed into protein compounds that may be consumed by humans. Microbial communities in the rumen employ forage protein metabolic byproducts as key components for the synthesis of other proteins, which are then used to make milk and meat. Leguminous protein broke down very easily in the rumen such as alfalfa, where hemicellulose, cellulose (cell wall component of plant) comprises as a main energy source are processed at a slow rate. Unfortunately, there is an oversupply of fodder proteolysis byproducts and a deficit of cellulose and hemicellulose breakdown products, which give the fuel for protein biosynthesis, the microbial activity is confined in terms of energy for protein biogenesis (Kingston-Smith et al., 2003). As a result, plenty of extra nitrogen is discharged onto

grazing land in urine, contributing to nitrogen reaching up to 70 percent (Kingston-Smith et al., 2013) and a significant economic influence on consumers. (Moorby et al., 2009). Not only ruminants but also chickens, turkeys, horses use alfalfa because of its high nutrition value. (Schmidt & Singh., 2009).

2.5 Citrus Based by Products as a Feed Stuff

Fresh citrus pulp, citrus silage, citrus molasses, dried citrus pulp, citrus powder, lemon peels, and citrus-activated sewage have all been used as substitute feeds for ruminants at various phases of development. The Mediterranean countries such as Spain, Italy, Turkey, Morocco, Egypt, and Greece produce about 24 percent of the world largest citrus, with Brazil (24%) and the United States (21%) becoming significant capital citrus producers (Ammerman and Henry, 1991). Citrus by -product comprises sugars (glucose, fructose, sucrose), bitter principles (isolimonin, limonin), flavonoids (hesperidin, naringin), acids , lipids (linolenic, oleic, palmitic, linoleic, stearic acids, phytosterol, and a glycerol), Nitrogen (1–2 g/kg on a wet basis), enzymes (pectinesterase, phosphatase, peroxidase), vitamins (Vitamin B complex, ascorbic acid, carotenoids), pigments (xanthophylls and carotenes), minerals (primarily calcium and potassium) and peel oil (d-limonene). Citrus BPF are appropriate for enclosure in ruminants feed because rumen can ferment high fibrous feed (Grasser et al., 1995).

By-products of citrus could play a key role in resolving this critical matter. Feeding citrus by-products to domesticated animals comes in a variety of formats (Bampidis and Robinson 2006). Raw by-product of the citrus juice business is fresh citrus pulp (FCP), which contains all of the components of the citrus fruit except the extracted citrus juice (Hutton 1987). It is appealing to animals, has a low cost in comparison to other feedstock with similar nutritional benefits, and contains digestible elements. It is also high in antioxidants and vitamin C, which has a favorable impact on the animal's quality and development.

The drawbacks of FCP include bulkiness, the fact that it spoils fast and becomes a flight problem if let to decay, and the fact that it has an uneven Ca: P ratio. Citrus pulp's utility could be increased by ensiling or dehydrating it and turning it to dry citrus pulp due to its high-water content 76-87% (Bakr, 2015). Citrus pulp Ensiling is a fantastic way to keep

it usable all year long (Fuller, 2004). Cattle quickly accept the silage because it has a pleasing odor. Citrus pulp improves the quantity and the quality of silage (lower pH, hogh sugars and acidic bacteria) while reducing a requirement for acid supplements (Crawshaw, 2004). Without the use of a supplement, fresh citrus pulp silage can be inspected and maintained (Revuelta et al., 2008). Furthermore, citrus pulp ensiled without chemicals has a higher energy conversion as evaluated by volatile fatty acid synthesis (Itavo et al., 2000).

After extracting the juice from fresh fruits and drying the leftovers, the dried citrus pulp is generated as a by-product. It's defined as a by-product meal made from energy concentrates. The dried citrus pulp (DCP) has been used to replace grains (corn, barley, and wheat) in various amounts, as well as antioxidant feed and beet pulp supplements. Citrus pulp is normally given dried, but it should be given access into a ration to enable the ruminants to acquire acclimated to its distinctive aroma and flavor (Bath et al., 1980). Citrus pulp, on the other hand, can be feed fresh or as silage. Both can be readily taken by ruminants, however lemon peels and pulp are slightly more palatable than orange and grapefruit peels and pulp (Bath et al., 1980). Citrus BPF appears to have no effect on ruminant consumption in diets that contain it. For example, in the concentrate fed to Friesian dairy cattle, incomplete or entire replacement of barley or corn grain with dried dried lemon pulp (DLP) and orange pulp (DOP) had no influence on fodder consumption (Lanza, 1984).

At up to 400 g DCP/kg dry matter, the consumption of a ration provided to Awasi lambs having DCP was observed to be much like that including maize grain, but fell at greater levels (Bhattacharya and Harb, 1973). Whereas, increasing N content by ammoniating 450 g DCP/kg dry matter with or ammonium hydroxide urea had little effect on sheep palatability (Rihani et al., 1993b). Furthermore, Volanis et al. (2004) observed that composted chopped oranges at 309 g/kg DM of the TMR were digestible to lactating dairy sheep, apparently due to the fresh scent, whereas Migwi et al. (2001) suggested that the value of citrus pulp in sheep rations should be kept between 150 and 200 g/kg DM to avoid depressed consumption. Finally, orange has been utilized to flavor sheep's food (Ralphs et al., 1995).

2.6 Status of By-Products in Turkey

Turkey has an area of 78.35 million hectares (MH) and is located between Europe and Asia. For the past 2 decades, the total amount of agricultural land has been steadily declining. A total of 37.80 MH is used for agriculture, with 18.93 MH for grains and other crop products, 0.784 MH for greenhouses, 0.005 MH for ornamentals, 3.462 MH for fruit, drinks, and medicinal crops, and 14.62 MH for perennial pastures and grasslands (TUIK, 2018). Livestock farming accounts for around 30% of Turkey's agriculture industry.

During grazing seasons, most animals rely on grazing lands and harvest wastes for food. Grasslands are especially vital during crop growth season, when artificial meadows and feed resources are scarce and substantial animal rearing is required. There are 17,042,506 cattle and 46,117,399 ruminants in Turkey's bovine population (TUIK, 2018). The number of cattle has remained stable, whereas the number of small ruminants has declined dramatically between 1985 and 2010. By 2010, the numbers of small ruminants began to rise and is now constantly rising. Turkey's maximum elevation is around 1000 meters, and the livestock feeding phase is just 180 days long (Altin et al., 2011).

Approximately 7.5 million animal units acquire their food from grasslands, and their feedstuffs need is around 13.5 million tons (MT). Grasslands in Turkey contribute roughly 7.6 MT of feed ingredients; however, this amount is insufficient to meet animal demands. (Koc et al., 2012). During the foraging season in Turkey, there is a big imbalance between market forces for feedstuffs. Poor-quality feed residues, fallow land, and underbrush flora are used to meet this requirement. In the intensive program, around 2.65 million AU ruminants, primarily cattle, are kept, with feedstuffs demands reaching 4.75 MT during the summer period (TUIK, 2018). In the winter, however, 18.75 MT of roughages are needed, with a total fodder requirement of 25.5 MT for extensive breeding systems. In the country, total hay output is around 13.3 MT. In Turkey, there is an annual roughage difference of 12 MT between spring and autumn. Alternative fiber sources include vegetable leftovers, sugar beet pulp and leaf, and the forest floor of fruit gardens, which contribute for roughly 5.0 million metric tons. Eventually, cereal straw fills in the fodder shortfall with 7.2 MT (Koc et al., 2012).

2.7 Rumen Fermentation and Digestion in Ruminants

Ruminants have developed a large pre-gastric fermentation framework with four chambers (rumen, reticulum, omasum, and abomasum) in which microbes can degrade highly complex polysaccharides into molecules that can be digested by the animal. Because of this adaption, forages grown on the wide grasslands acres around the globe can be used. Ruminants generate half of the beef and practically all the milk. Forage fermentation predominantly in the ruminant's reticulo-rumen section of the induced gastric tract. Food goes from the reticulo-rumen section of the gut to the omasum, where functionality is unknown but where certain degradation product is absorbed, then on to the abomasum, which is identical to the regular abdomen of monogastric. Rumen grows quickly, eventually accounting for 80 percent of the stomach's capability. (Campbell and Lasley, 1969). Three-quarters of the abdomen is taken up by the stomach (Church, 1969). Ruminants only swallow long enough just to combine the feed with saliva and produce a flush. When the animal recycles the bolus during rumination, more swallowing happens. Ruminants regurgitate their ingesta, ingest the regurgitated fluids, re-masticate the solids via re-insalivation, and re-swallow the bolus to lessen particles. Rumination impacts the digestion of feeds because it enhances digestion by lowering grain size, which impacts the median duration feed spends in the rumen. The ruminant’s digestibility is a complicated relationship between the animal, the feed, and the microbiota. Fluid flow in the rumen could be divided into three categories: fluid, particle big enough just to go through rumen, and particle retained in the rumen and they're too larger to pass by via omasal opening. The ingesta floats on the rumen in rather large particles. Rumination takes place on the larger fragments, which are then broken down into smaller bits. The ingesta moves into the rest of the gastrointestinal tract after interaction to the bacteria populations and rumination, which decrease molecular weight. The rumen microorganisms decompose most of the forage's fermentable sugars and degrade 40-80% of the protein consumed. Lactic acid, volatile fatty acids, CO_2, CH_4, microbial protein, and bacterial polysaccharide are all constituents of ruminal fermentation. The volatile fatty acids being taken into the bloodstream through rumen wall and used in metabolic activities. Lactic acid, microbiological protein, and bacterial cellulose move through the rest of the digestive system, where they are digested and absorbed. Eructation is a mechanism that allows methane and carbon dioxide to be ejected orally.

The ruminant digestive system has the effect of retaining the fundamental elements of the ingesta in the rumen for an extended time. According to Bell (1971), the ruminant digesting approach is to increase the effectiveness of protein utilization at the price of the ample source of energy. Whenever the dietary contents of cell walls (complex structural polysaccharides) are substantial, the ingesta is retained in the rumen for longer to confirm derivation. With most forages, cattle have a 50–60-hour storage duration in the rumen. Grassy dicots have short processing durations than herbaceous dicots. Low fiber, lignin diets accelerate the pace of ingesta transit through the induced gastric tract. Ruminant feedstuffs ingestion is controlled by rumen ability. The bigger the intake, the faster the rumen discharges. The rate of passing is determined by the bioavailability of the forages. The far more palatable a feed is (fiber, lignin content and low cell wall), the faster it passes through the digestive system, leading to increased intakes. Because of the mechanism of ruminant digestion, the ruminant cannot adjust for a relatively low diet by digesting more fodder. In ruminants, the amount of ingesta digested reduces as the N content of the food falls and fiber constituent concentration rise, whereas excreta rise. This mixture is placed straight into the food web of decomposers. An ecosystem disturbance that affects fodder quality affects the absorption factor, which is the source of productivity in the form of growth and/or breeding. The following are a few previous studies papers on the use of citrus-based products to improve ruminant digestibility and fermentation.

Tayengwa et al. (2021) studied the utilization effect of dried citrus pulp (DCP) and grape pomace (GP) on digestibility and nutrient intake in steers. Twenty-four steers were treated in a complete randomized design and were fed with three types of diets. It was noticed that steers fed by GP were having high dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF) and starch intake compared to DCP and control diets. There was higher digestibility of DM, OM, and NDF compared to DGP and control diets. The steers fed with control diets were having higher urinary excretions of uric acid, purine and allantoin derivates than DCP and GP. The findings of the study suggested that GP maybe a better fiber substitute for wheat bran, but DCP have high apparent nutrient digestibility.

Ahmed et al. (2021) investigated the impacts of citrus and garlic extraction on feed consumption, digestibility and fermentation and in sheep. The experiment was performed by 21 days interval, by recording weight weekly and health status of sheep daily. The

results of experiment showed that garlic and citrus supplementation didn't affect the total feed intake, growth performance or digestibility and rumen fermentation. Compared to control treatment, the treatments reduced the methane discharge yield per digestible DM ingestion in the rage of 7 %-12.8 %, correspondingly, while there was no effect on CO2 emission. In conclusion, a mixture of citrus and garlic extracts, is a potential normal feed additive that can be utilized to decrease the methane discharges from ruminants exclusive of creating any negative impact total feed intake, digestibility and fermentation or growth performance and thereby leads to improved animal health.

Sharif et al. (2018) conducted a research study to determine the effect of dried citrus pulp (DCP) as a partial concentrate replacement in small ruminants by using chemical analysis. 49 Ruminants were separated by RCBD design. 70:30 ration was fed to lambs. Four treated samples with changed ratio 10,20,30,40% DCP were continued for 4 months. As a result, it showed that 40% of DCP with concentrate feed is favorable for animal performances.

Besharati et al. (2017) estimated the effect of orange pulp, molasses, and *Lactobacillus buchneri* supplementation to alfalfa silage on gas production and *in vitro* dry matter digestibility by using 6 treatments including, alfalfa hay with bacterial additive, alfalfa hay with orange pomace, alfalfa hay with orange pomace and bacterial additive, alfalfa hay with molasses and alfalfa hay with molasses and bacterial additive and alfalfa hay (control). Alfalfa hay mixed with orange pulp ensiled for 90 days. After 24 hours in incubation, silage ALFO (alfalfa + orange pulp) and control (without additive) and silage ALFO (alfalfa + orange pulp) after 24h incubation showed the lowest and highest *in vitro* gas production. *In vitro* DM digestibility reduce with the addition of additive. This shows that alfalfa+ orange pulp silage and molasses can progress silage value and improved *in vitro* DM digestibility.

Lashkari et al. (2014) performed a study to examine the digestion kinetics of citrus by-products carbohydrate components. Tested samples were orange pulp (OP), lemon pulp (LP), Grapefruit pulp (GP), and lime pulp (LI). The *in vitro* gas production technique was applied to measure digestion kinetic, neutral detergent fiber (NDF) and acid detergent fiber (ADF) while for fermentation kinetic, curve subtraction was used. LE had highest fermentation rate and tested feed were showed no difference in gas production. LE and

LI exhibited higher dry matter digestibility values and lower for acid detergent fiber (ADF). However, higher digestibility rate of NDF showed in GP and LI. This study concluded that citrus carbohydrate fraction has high degradability potential and notable difference were also observed among digestive behavior and digestion kinetic.

Arbabi et al. (2010) evaluated the treated silage of orange by-products in the form of dried sugar beet pulp (DSP) and dried citrus pulp (DCP), and wheat straw (WS) in mini silos by using proximate analysis. By using Flieg,s method the DCP and DSP silages showed better apparent quality than control silages. Whereas there was no difference in pH in DCP and DSP silages as compared to control. Treated silage with DCP showed lower NDF, but high CP and DM as in DSP whereas, wheat straw silage had high ADF. As a result, this experimental study suggested that citrus pulp silages are beneficial for animal production.

Rofiq and Gorgulu, (2014) studied the effect of orange peel oil and clove by using Ankom Daisy^{II} Incubator on *in vitro* digestion of Dairy Total Mixed Ration, for rumen manipulation. The outcomes showed that orange peel oil reduced IVTDMD, IVNDFD and energy estimate of total mixed ration whereas, clove maximize it. On the other hand, in a combination they are incompatible to decrease invitro digestion of total mixed ration.

Silva et al. (1997) investigated the digestion kinetics of citrus peel in the rumen of cows fitted with rumen canulae. Three experiments were done 1) silage of orange peel 2) fresh peels from two lemon + orange peel 3) silage of sugar cane and orange peel arranged in blend with broiler litter. The control treatment used was the corn silage. Samples were dried at 50°C, then suspended in the rumen at 48 h of incubation. Digestibility was similar for citrus peel silage or as fresh, ranging from 51- 58% at a discharge rate of k = 0.05. Calcium hydroxide supplement to peels exhibited some undesirable effect on ruminal digestibility. All citrus peels were showing higher capability of degradable dry atter in the rumen which represents the high energetic quantity for cattle.

Lima et al. (2014) evaluated the impact of supplementation of citrus pulp and soybean oil on ruminal fermentation and true tract apparent digestibility (TTAD). It was noticed the citrus pulp and soyabean lowered the basal DM intake compared to those fed with soyabean oil only whereas the ruminal pH and the concentration of ammonia was not

affected. Also, the treatments have no effect on DM and NDF. Citrus pulp + soyabean oil had minor effects on ruminal fermentation and true digestibility characteristics comparatively. There is no need of citrus pulp supplementation as a supply of antioxidants to avoid rumen when cows were fed with unsaturated fatty acids as soyabean oil.

Villarreal et al. (2006) checked the supplementation effect of pelleted citrus pulp (PCP) on intake and digestibility of tropical grass-based diet in beef cattle. Stargrass (*Cynodon nlemfuensis*) was daily harvested and copped and were fed as a basal diet. The increasing amount of PCP supplementation linearly increased the degradability of organic matter and dry matter but the digestibility of forage DM and NDF were not affected. Mean value of pH was in the range of 6.6-7.2 which was not affected by supplementation. It was concluded that high levels of PCP supplementation can decrease the forage intake but increase the energy intake and could be helpful in combination with forages.

CHAPTER III

MATERIALS AND METHODS

3.1 Attainment and Preparation of Material

The *In vitro* digestibility of alfalfa silage supplemented with different amount of orange and mandarin pulp were determined in the animal nutrition laboratory of the Ayhan Şahenk Faculty of Agricultural Science and Technologies in Niğde Ömer Halisdemir university, Turkey. The whole first cut Alfalfa (*Nimet spp*) was harvested beginning of bloom and wilted to at room temperature for 24 hours. Citrus *spp*. was bought from local market in Nigde. The wilted Alfalfa and fresh orange (*C. sinensis*) and mandarin (*C. reticula*) pulp were sliced manually to an approximately 2 cm theoretical length of cut.

3.2 Preparation of Citrus Pulp

Citrus pulp (CP) is the left-over residue after citrus fruits are processed for juice or other products. All citrus samples taken were in good quality. A total of 15kg of orange and 15kg of Mandarin were purchased and used for pulp preparation. The pomace was made up of the peels and the pulps. The fruits were washed and cut into half with a kitchen knife. A manual juice extractor was used to extract the juice from the citrus peels. The pulp was then cut into small pieces of about 1-2 cm theoretical length of cut and then placed in a labeled nylon bag. Each citrus sample was placed in a pre-weighed aluminum dish (tare) and weighed using an electric weighing scale with four digits.

3.3 Preparation of Silages

Ensiling is a feed preservation technique based on a spontaneous lactic acid fermentation in anaerobic conditions. 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 the preparation of silage in October 2020, fresh alfalfa was collected from the field of Niğde Ömer Halisdemir, University. The Alfalfa were spread uniformly on the clean sack

on concrete floor for wilting under shade for 2 days to reach the moisture level better for silage. Orange and Mandarin pulp were added to alfalfa harvested at beginning of flowering stage and wilted for 24 hours with a ratio of 0.20, 0.25, 0.30 and were ensiled for 60 days. The treatments included: 1) alfalfa hay (control); 2) alfalfa hay with orange pulp; 3) alfalfa hay with mandarin pulp.

1) ALF-Hay: Alfalfa hay-wilted (control)
2) ALFO 20: Alfalfa hay-wilted 0.80 + orange pulp 0.20
3) ALFO 25: Alfalfa hay-wilted 0.75 + orange pulp 0.25
4) ALFO 30: Alfalfa hay-wilted 0.70 + orange pulp 0.30
5) ALFM 20: Alfalfa hay-wilted 0.80 + mandarin pulp 0.20
6) ALFM 25: Alfalfa hay-wilted 0.75 + mandarin pulp 0.25
7) ALFM 30: Alfalfa hay-wilted 0.70 + mandarin pulp 0.30

The weighed samples were placed in the oven at a temperature of 50^{o}C for 48 h. After 48 h in the oven, they were removed and placed on the bench for 24 h to cool. The samples were weighed and grinded using a 1mm sieve at a speed of 1400 rpm by Rechts ZM200 grinder. They were then stored in pre-labelled plastic bags prior to chemical analysis.

Figure 3.1. Preparation of silages

3.4 Chemical Analysis

The dry matter content (DM), Crude ash (XA) and Crude protein (CP) of wilted alfalfa and citrus pulps and silages after 60 days of ensiling was determined. Wilted alfalfa and citrus pulps and silages after 60 days of ensiling were analyzed for dry matter (DM), according to the AOAC (1995). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and CF contents of citrus pulps, wilted alfalfa and silages were analyzed by fiber analyzer ANKOM 200 using the method of VAN Soest (1991) and all chemical analysis were carried out in triplicates.

3.4.1 Dry matter determination

Crucibles were cleaned and oven dried for 2 h to a constant weight (W1). 1 g from mandarin and orange pulp silage + alfalfa was weighed using an analytical balance and recorded as (W2) and placed inside the pre-weighed crucibles. The samples were then placed in the oven with the aid of a tong and allowed to dry at a temperature of 105ºC for 24 h. After 24 h, they were taken from the oven and were placed in the desiccator for 20 min to cool to room temperature. The weight of the crucibles containing the samples (silages) were taken immediately and recorded as (W_3). The dry matter was then calculated in percentage using formula (3.1).

$$\%\ Dry\ matter = \frac{(W3-W1)}{W2} \times 100 \quad (3.1)$$

3.4.2 Crude ash

Crude ash estimation was done using a muffle furnace at 600ºC for 4 hours. One gram of sample was taken into clean dried (105ºC for 8 hours) crucibles and were placed in the muffle furnace. After burning, the crucibles were transferred into desiccator to let it cool. Crucibles were reweighed and crude ash were calculated using the following formula (3.2).

$$\%\ Ash\ content = \frac{(W4-W1)}{W2} \times 100 \quad (3.2)$$

Figure 3.2. Muffle furnance

3.4.3 Crude protein

Crude protein (CP) of the samples was estimated using the Kjelroc digestion unit and Opsis liquid line Kjelroc analyzer made in Sweden. 1g each of orange and mandarin silages were put into an Opsis tube in triplicates. Two kjedahl tablets and 12 mL of H_2SO_4 was added into each tube. The tubes were placed in the digester block and the protein digester was set at 420^oC for 60 min. The suction was regulated to neutralize the fumes that were released during the digestion process. After the heating period was completed, the block cooled for 15 min.

The protein content was then estimated using the Opsis liquid line Kjelroc analyzer through distillation and titration. Large kegs of distilled water, 40% sodium hydroxide solution, and 1% boric acid with mixed indicators (100 mg bromocresol green and 70 mg methyl red dissolved separately in 100 mL methanol) were attached to Kjelroc Analyzer. The machine was auto cleaned, and blank was determined before analyzing the samples for crude protein.

The Kjedahl program was set at % protein with a nitrogen factor of 6.25 i.e., **Crude protein** = N×6.25. The weight of sample (1g) in each tube was imputed into the machine display screen and the tubes with corresponding weights were analyzed for crude protein. The crude protein value for the sample in each tube was displayed on the screen on completion of titration and distillation. These values were recorded as crude protein content for the samples.

Figure 3.3. Opsis liquid line and Kjelroc analyzer

3.4.4 Neutral detergent fiber

Neutral detergent fiber (NDF) was estimated using ANKOM 200 fiber analyzer. Twenty-one F57 filter bags were labelled with a solvent resistant marker and then weighed (W1) with an analytical balance. The balance was zeroed and 0.55 g each of silage sample was weighed directly into the filter bag. The bags were tapped gently for uniform spreading of samples and elimination of clumping. The bags were then sealed within 4 mm of the top using a heat sealer. One blank bag was also weighed to include in the run for determination of blank bag correction (C1).

NDF solution was prepared using, 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 mixed in 1 L distilled H_2O to make

up one liter solution. 20 g of Sodium sulphite (Na2SO3) and alpha amylase was also used in the extraction process. The bags were putted into the bag suspender. Three bags were arranged per tray and the trays were stacked such that each tray was rotated at 120^{o}. The bag suspender was then inserted into the fiber analyzer vessel and the suspender weight was placed on top to keep the bag suspender submerged. 2000 mL of ambient ND solution was prepared and poured into the fiber analyzer vessel. 20g of Na_2SO_3 and 4.0 mL of alpha amylase was added to the solution in the vessel. To start the digestion process, the agitate and heat button was turned on. Agitation was confirmed and the lid was closed. The timer was set to 75 min. At the end of extraction, the heat and agitate button was turned off and the drain valve was released to allow exhaust of the hot solution before the lid was opened.

After releasing the solution, 2000 mL of water at 90^{o}C with 4.0 mL of alpha-amylase was added to the vessel for the first and second rinses. Each rinse was for 5 min. Tap water without alpha-amylase was then used for the third rinse which was also for a 5 min duration. On completion of the rinsing process, the samples were removed, and the bags were tapped gently to remove excess water. The bags were soaked in acetone for 5 min and then completely air-dried for 20 min on filter paper. The bags were then placed in the oven to dry completely at 105^{o}C for 2 h after which, the bags were directly placed into a collapsible desiccant pouch for 20 min to cool to ambient temperature. With the use of an analytical balance, the bags were weighed (W3) and percentage NDF (as received basis) was calculated using formula (3.3).

$$\% NDF = \frac{W3-(W1\times C1)}{W2} \times 100 \quad (3.3)$$

Where W1=Bag tare weight,
W2 = Sample weight,
W3 = Dried weight of bag with fiber after extraction process,
C1 = Blank bag correction (final oven – dried weight divided by the original blank bag weight).

3.4.5 Acid detergent fiber

Acid detergent fiber (ADF) was also estimated using ANKOM 200 fiber analyzer. Twenty-one F57 filter bags were labelled with a solvent resistant marker and then weighed (W1) with an analytical balance. The balance was zeroed and 0.50 g (W2) each of silage sample was weighed directly into the filter bag. The bags were flicked gently for uniform spreading of samples and elimination of clumping. The bags were then sealed within 4 mm of the top using a heat sealer. A blank bag was also weighed to include in the run for determination of blank bag correction (C1).

The bags were placed on the trays in the bag suspender with three bags arranged on each tray. The trays were stacked such that each tray was rotated at 120°. All the nine trays were placed in the bag suspender and the bag suspender was then inserted into the fiber analyzer vessel. The suspender weight was placed on top to keep the bag suspender submerged. ADF solution was prepared using, 20 g cetyl trimethylammonium bromide (CTAB) with 1 L of 1.00N H_2SO_4. 2 L of ADF solution was poured into the fiber analyzer vessel and the lid was closed. The digestion process was set for 75 min, and it was started by pressing the agitate and heat button. On completion of the digestion process, the solution was released through the exhaust pipe. The samples in the vessel were rinsed with hot water twice for 5 min each time. A third rinse was carried out using tap water also for a 5 min duration. After the rinse, the bags were removed, and excess water was pressed out from the bags by gentle tapping. Following this, the bags were soaked in acetone for 5 min after which they were removed and completely air-dried for 20 min on filter paper.

The bags were then placed in the oven for 2 h at 105° to dry completely. After 2 h, they were removed from the oven and were immediately placed in a collapsible desiccant pouch for 20 min to cool to ambient temperature. The bags were weighed W3 and percentage ADF (as received basis) was calculated using Equation (3.4)

$$\%\ ADF\ (as\ received\ basis) = \frac{W3-(W1\times C1)}{W2} \times 100 \qquad (3.4)$$

Where W1 = Bag tare weight

W2 = sample weight

W3 = dried weight of bag with fiber after extraction process

C1 = Blank bag correction (running average of final oven- dried weight divided by original blank bag weight).

3.5 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).

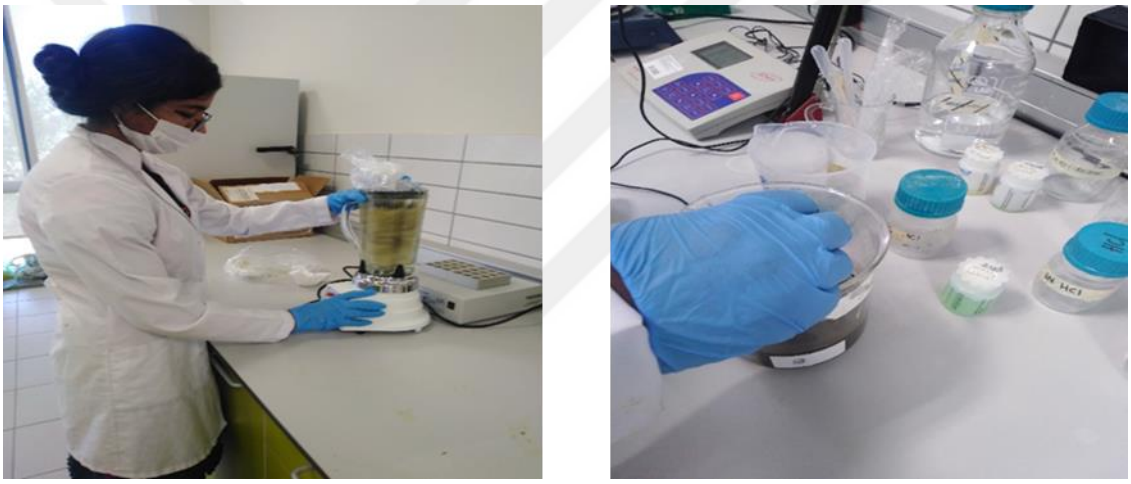

Figure 3.4. Measurement of pH of samples

3.6 *in-vitro* True Digestibility

In-vitro true digestibility (IVTD) of the two species of orange and mandarin pulps + Alfalfa samples were determined using DAISY II incubator by ANKOM Technology (Macedon, NY). Daisy II Incubator has four incubation jars with a capacity of 2000 mL each. 1600 mL buffer solutions and 400 mL rumen liquor were added into each jar. The filter bags were placed in the jars.

3.6.1 Preparation of filter bags and samples

Ankom F57 filter bags were rinsed in acetone for 3 min and completely air-dried. Each bag was then weighed, and the weight was recorded as (W1). The balance was zeroed, and 0.5 g of sample (W2) was weighed directly into the filter bag. Empty filter bags were weighed and sealed as blank for correction factor. Afterwards, the bags were heat sealed using an impulse bag sealer. The experiment was run in two batches with triplicates of each of the twenty-one silage samples (Alfalfa, Orange and Mandarin).

3.6.2 Preparation of (combined Buffer Solution A and B)

Buffer A and Buffer B solutions were prepared a night before the incubation using the reagents and concentrations in Table 3.1. A volume of 266 mL of solution B (Basic solution) was added to 1330 mL of Solution A (Acidic solution). The exact amount of solution A to be added to solution B was adjusted with the use of a pH meter to obtain a final pH of 6.8 at 39^{o}C.

Table 3.1. Buffer A and B solutions for *in vitro* digestibility trials using Ankom Daisy^{II} incubator

Reagents	**g/L**
(a) Buffer Solution A	
KH_2PO_4	10
$MgSO_4.7H_20$	0.5
NaCl	0.5
$CaCl_2.2H_20$	0.1
Urea (reagent grade)	0.5
(b) Buffer Solution B	**g/L**
Na_2CO_3	15
$Na_2S.9H_20$	1

continued

3.6.3 Preparation of Inoculum

The rumen liquor (inoculum) was collected postmortem from the rumens of two mature Holstein cattle that were slaughtered in a commercial abattoir in Niğde. Two 2000 mL thermos flask preheated at 39^{o}C and purged with CO_2 was used to store the collected rumen liquor. Two fistfuls of fibrous mat from the rumen of the cattle were added to one of the flasks after which the thermos flasks were taken to the laboratory. The rumen liquor was poured into a blender that was preheated to 39^{o}C. The liquor was purified with CO_2 gas for 30 sec and then blended at a high speed for 30 sec. After that, the digesta was filtered through four layers of cheesecloth into a five-liter flask that had been prepared to 39 degrees Celsius. The remaining rumen fluid in the other thermos was similarly filtered into the same five-liter flask via four fresh layers of cheesecloth. The rumen digesta in the five liters flask was continually purged with CO_2 until inoculum was transferred into the digestion jars.

3.6.4 Incubation of samples

The resultant Buffer solution (A and B) (1600 mL) and rumen inoculum (400 mL) were poured into each daisy II digestion jar after which each digestion jar was purged with CO_2 for 30s. The Filter bags with sealed blank bags for correction factor (C1) and were then evenly distributed into three daisy II jars. The digestion jars were purged with CO_2 and tightly sealed. All the digestion jars were placed into the pre-heated Daisy^{II} incubator and incubated for 48h at a temperature of 39^{o}C±0.5. After 48 h of incubation, the bags were removed from the digestion jars and rinsed with minimal mechanical agitation with cold tap water until water was clear to stop the microbial activity. The bags were placed in sealable bags and stored in the refrigerator before NDF determination. ANKOM200 fiber analyzer was used to determine NDF of the samples and the weight after determination of NDF was recorded as W3. The percentage in-vitro true digestibility on as received and dry matter basis was then calculated using formulas (3.5) and (3.6).

$$\%\ IVTD\ (as\ recieved\ basis) = 100 - \frac{W3-(W1\times C1)}{W2} \times 100 \quad (3.5)$$

$$\%\ IVTD\ (DM\ basis) = 100 - \frac{W3-(W1\times C1)}{(W2\times DM)} \times 100 \quad (3.6)$$

Where W1 = Bag tare weight

W2 = Sample weight

W3 = Final bag weight after in-vitro and sequential ND treatment

C1 = Blank bag correction (final oven-dried weight/original blank bag weight)

3.6.5 Neutral detergent fiber

Neutral detergent fiber (NDF) of the digested samples was estimated using ANKOM 200 fiber analyzer. The bags containing the digested samples were placed into the bag suspender. Three bags were arranged per tray and the trays were stacked such that each tray was rotated and properly placed. The bag suspender was inserted into the fiber analyzer vessel and the suspender weight was placed on top to keep the bag suspender submerged. 2000 mL of ambient ND solution was prepared and poured into the fiber analyzer vessel. 20 g of Na_2SO_3 and 4.0 mL of alpha-amylase were added to the solution in the vessel.

To start the digestion process, the agitate and heat button was turned on. Agitation was confirmed and the lid was closed. The timer was set to 75 min interval. At the end of extraction, the heat and agitate button was turned off and the drain valve was opened to allow exhaust of the hot solution before the lid was opened. After releasing the solution, 2000 mL of water at 90^{o}C with 4.0 mL of alpha -amylase was added to the vessel for first and second rinses. Each rinse was for 5 min interval. Tap water without alpha-amylase was then used for the third rinse which was also for a 5 min duration.

On completion of the rinsing process, the samples were removed, and the bags were tapped gently to remove the excess water. The bags were soaked in acetone for 5 min and then completely air-dried for 20 min on filter paper. The bags were then placed in the oven to dry completely at 105^{o}C for 2 h after which, the bags were directly placed into a collapsible desiccant pouch for 20 min to cool to ambient temperature. With the use of an

analytical balance, the bags were weighed (W3) and percentage NDF (as received basis) was calculated using formula (3.7).

$$\%\ NDF\ (as\ recieved\ basis) = \frac{W3-(W1\times C1)}{W2} \times 100 \qquad (3.7)$$

Where W1=Bag tare weight

W2 = Sample weight

W3 = Dried weight of bag with fiber after extraction process

C1 = Blank bag correction (final oven – dried weight divided by the original blank bag weight)

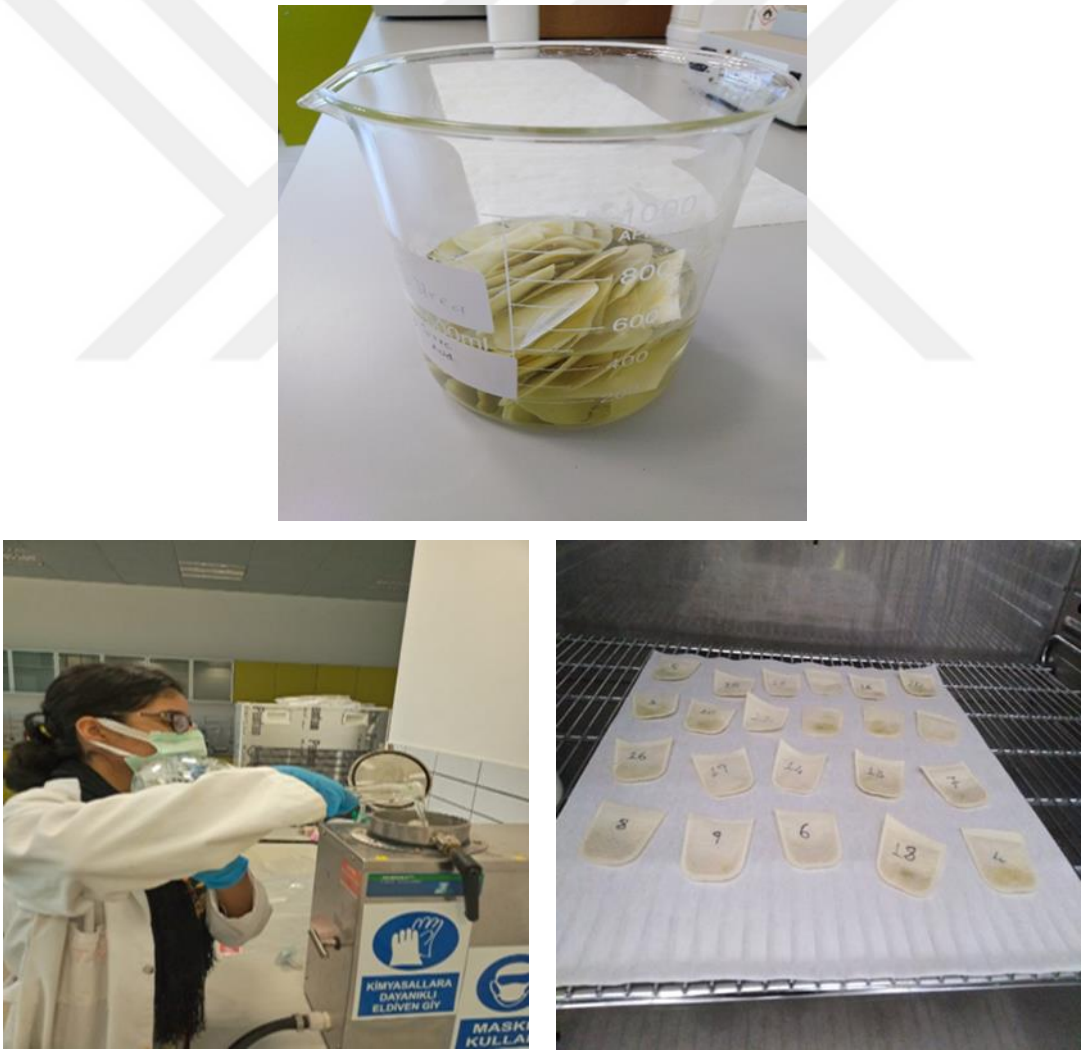

Figure 3.5. Fiber Ankom Analyzer

3.7 Statistical Analysis

The results of chemical parameters were subjected to Kruskal-Wallis' non-parametric one-way ANOVA to make sure statistical validity of normality, homogeneity, and independence for this study, while Tukey's multiple comparison test for ANOVA was used to evaluate the effect of alfalfa + citrus (orange and mandarin) silages on in-vitro digestibility. Jamovi, a statistical analysis tool, was used for all of the analyses. P 0.05 was used to determine statistical significance, and the findings of these analyses were presented in the form of tables and graphs.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Chemical Composition of Alfalfa, Orange, and Mandarin

The chemical composition of alfalfa-hay, mandarin and orange pulp samples were evaluated during the research study and were presented in table 4.1. The results showed that alfalfa-hay had higher (29.85±0.93) air dry matter (ADM%) followed by orange (24.11±0.23) and mandarin (22.63±0.28). Highest percent dry matter (DM%) were recorded for alfalfa-hay (94.56±0.15), followed by mandarin (92.25±0.81) while the lowest DM% were recorded for orange with value of (91.13±1.22). Ash content (XA) was observed higher (7.67±0.31) in alfalfa-hay than mandarin (4.13±0.00) and orange (3.36±0.09). The alfalfa-hay had greater (18.73±1.03) crude protein (CP) content followed by mandarin (6.25±0.05) whereas, orange had lower (5.77±0.05) crude protein (CP) content among the tested samples. The orange and mandarin samples (34.02±6.74 and 32.17±2.44) showed comparatively less amount of neutral detergent fiber (NDF) while the alfalfa-hay showed higher (34.22±2.07) neutral detergent fiber (NDF). The alfalfa hay samples were high (30.20±1.62) in acid detergent fiber (ADF) content where the acid detergent fiber (ADF) content was found low in orange (18.12±2.68) and mandarin (11.50±2.50). The pH values of alfalfa hay, orange and mandarin were (6.20±0.07), (4.85±0.06) and (5.56±0.09), respectively.

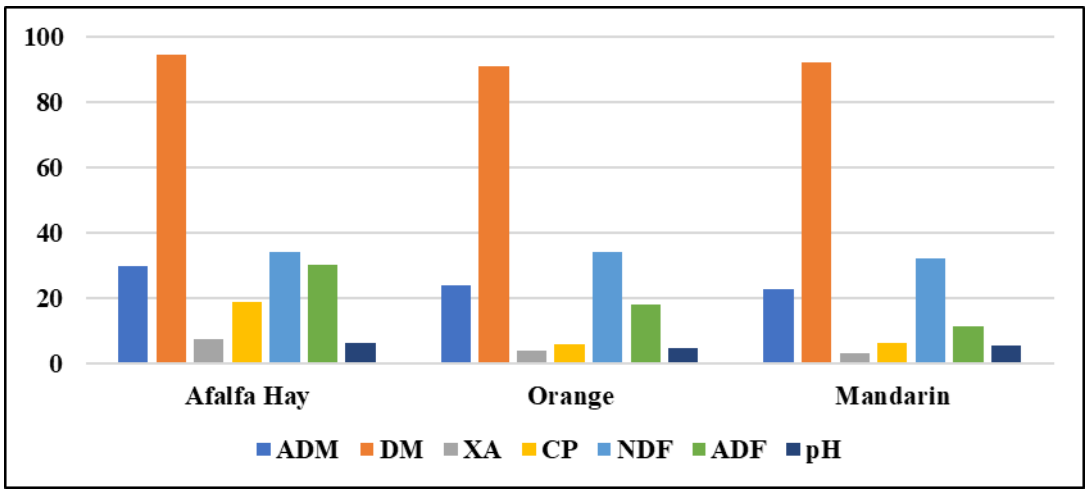

Figure 4.1. Chemical composition of alfalfa, orange, and mandarin

The dry matter (DM), ash (XA), crude protein (CP), NDF and ADF contents are the vital nutrients of a feed which determines the nutritive value of forages and alternative feed resources. Similar to our study Selçuk et al. (2019) have reported the DM% (93.20), Ash% (10.10), CP% (16.50) and NDF% (49.50) contents levels in alfalfa hay which are close to our results. The findings of Kamalak et al. (2004) are in accordance with our study who reported the DM% (91.52), Ash% (10.73), CP% (18.37), NDF% (42.40) and ADF% (27.36) content in alfalfa hay. Likewise, for chemical composition of alfalfa hay Rofiq and Gorgulu, (2014) has reported similar concentration of DM (92.80), Ash (8.21) and CP (14.73). For DM content in orange and mandarin Fegeros et al. (1995) has recorded same range of values for citrus *spp.* In the current study, the results for CP, NDF, and ADF in alfalfa hay are very close to the findings of Besharati et al. (2017). The chemical composition of orange and mandarin of this study is in similarity with the findings of Özkan et al. (2017) who reported CP, Ash, and ADF of 6.29, 4.43, and 15.86 for orange and 6.99, 6.87 and 14.59 for mandarin. Whereas the values for DM (23.90 and 21.12) and NDF (17.05 and 17.28) in orange and mandarin was in contrast to our findings. In terms of CP content, value of orange and mandarin are in the range with the findings of Villarreal et al. (2006) for peeled citrus pulp. Moreover, Besharati et al. (2017) examined the similar values of CP, NDF and ADF for orange as observed in our research study. Mínguez and Calvo, (2018) reported similar values of NDF and ADF in orange as compared to our study. Ash, CP, NDF and ADF content values recorded in the current study are in agreement with the values previously stated for mandarin by Kour et al. (2014). However, Besharati et al. (2017) reported the similar values of pH in alfalfa and orange pomace.

Table 4.1. Chemical composition of alfalfa, orange, and mandarin

Unit	Sample	ADM	DM	XA	CP	NDF	ADF	pH
1	ALF-Hay	29.85±0.93*	94.56±0.15*	7.67±0.31*	18.73±1.03*	34.22±2.07*	30.20±1.62*	6.20±0.07*
2	Orange	24.11±0.23	91.13±1.22	4.13±0.00	5.77±0.05	34.02±6.74	18.12±2.68	4.85±0.06
3	Mandarin	22.63±0.28	92.25±0.81	3.36±0.09	6.25±0.05	32.17±2.44	11.50±2.50	5.56±0.09

ADM = air dry matter, DM = dry matter, XA = ash content, CP = crude protein, NDF = neutral detergent fiber

ADF = acid detergent fiber

At $P < 0.05$ all the tested samples were not statistically different from each other.

* Shows the sample that ranked highest for each.

4.2 Chemical Composition of Alfalfa, Orange, and Mandarin Silages

The nutrient composition of the analyzed ingredients alfalfa, orange and mandarin silages are given in table 4.2. The samples of citrus (orange & mandarin) + alfalfa silages were compared with alfalfa silage (control). The highest value (29.37±0.54) of air-dry matter (ADM%) was found for ALFM30 (Alfalfa hay-wilted 0.70 + mandarin pulp 0.30) followed by ALFM25 (28.85±0.01) and ALFM20 (28.78±0.08), while the lowest (25.94±0.50) were found for control. Alfalfa silage (control) had the highest (94.59±0.27) percent dry matter (DM%) among the silage samples, while ALFM20 (Alfalfa hay-wilted 0.80 + mandarin pulp 0.20) had the lowest (91.42±0.90) among the same samples. On the other hand, the crude ash was significantly higher (14.40±0.06) in ALFO30 (Alfalfa hay-wilted 0.70 + orange pulp 0.30) than that ALFO20 (Alfalfa hay-wilted 0.80 + orange pulp 0.20). The value of crude protein (CP) content was found maximum (26.57±0.05) for ensiled alfalfa hay (control) whereas it was observed minimum (19.77±0.14) for ALFO25 (Alfalfa hay-wilted 0.75 + orange pulp 0.25). The NDF content in ALFO30 (Alfalfa hay-wilted 0.70 + orange pulp 0.30) were found low (36.46±1.14) however ALFM25 (Alfalfa hay-wilted 0.75 + mandarin pulp 0.25) had comparatively higher (39.96±1.35) NDF content amongst all treatments. Comparing control and mandarin + alfalfa silages, the ALFO20 (Alfalfa hay-wilted 0.80 + orange pulp 0.20) showed the higher (39.96±1.35) values of ADF whereas, lowest (25.54±8.97) was noticed for ALFO30 (Alfalfa hay-wilted 0.70 + orange pulp 0.30). The highest pH (5.70±0.10) was measured for AM25 (Alfalfa hay-wilted 0.75 + mandarin pulp 0.25) whereas low value of pH (4.12±0.01) was measured for AO30 (Alfalfa hay-wilted 0.70 + orange pulp 0.30), respectively.

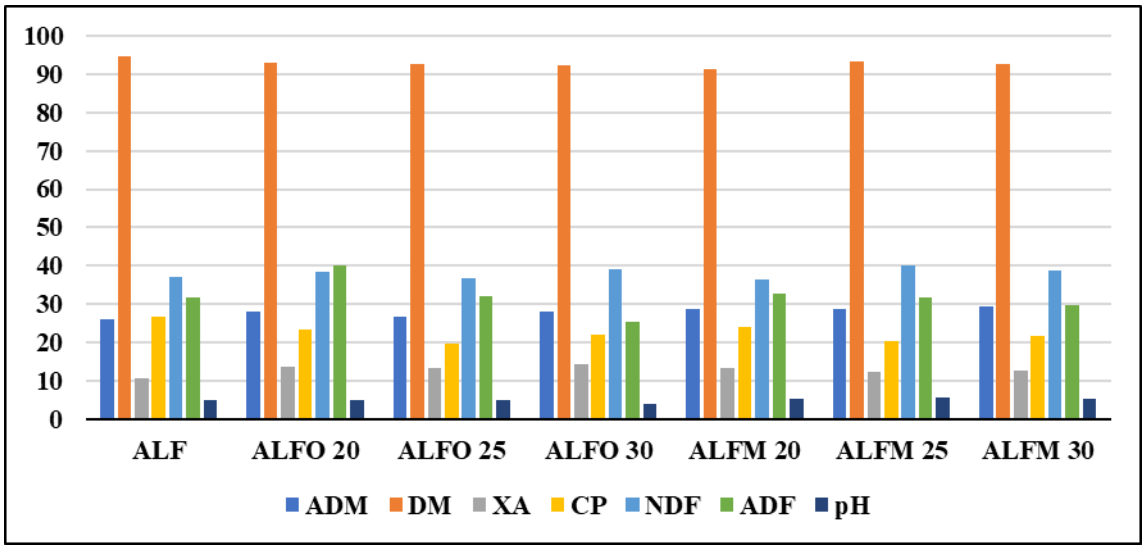

Figure 4.2. Chemical composition of alfalfa, orange, and mandarin silages

The increase in the dry matter content (DM%) due to supplementation of orange pulp possibly decreased the water activity of the ensiled silage mass that improved the fermentation process confirmed the hypothesis of the research study. Similarly, Grizotto et al. (2020) noticed the increase in dry matter content and stated that supplementation of pelleted citrus pulp to orange peel speeds up the fermentation process and decreases the losses in DM content as gas and effluent. The DM content of alfalfa remained the same as there were no additives that could have altered the fermentation activity. The results are similar with the findings of Gordon et al. (1961) who reported the DM content (89.9) for alfalfa hay silage. Ash content was enhanced by orange and mandarin addition, these results are in resemblance to the findings described by Ferrari Junior et al. (2009), who examined increased ash contents with the supplementation of 50 g kg^{-1} of citrus pulp and contrast with the findings of Gomes et al. (2017) stated that supplementation of citrus had no impact on ash content. Our results are supported by Ahn, (2007) who examined the chemical composition of citrus added with rice straw silage and stated that crude protein (CP%) content appeared enhanced with the addition of citrus to the rice straw silage related to control. Most Likely, the increase in crude protein concentration may possibly be due to soluble carbohydrate utilization through the fermentation process, that preceded to an indirect effect of protein concentration (Bernardes et al., 2005). Also, increases in CP content in silages were often noticed in orange peel silages (Valenca et al., 2016; Grizotto et al., 2017). In terms of NDF content, Kamalak et al. (2005) reported relatively close values to our findings for NDF and ADF in alfalfa silage. NDF is the structural component of a plant, because it provides bulk and is a predictor of voluntary ingestion

whereas ADF is the least digestible components of plants, including lignin and cellulose, so the feed with having low ADF content are mostly higher in energy. The results showed that ALFO30 (Alfalfa hay-wilted 0.70 + orange pulp 0.30) treatment increased the NDF content and reduced the ADF content thus enhanced the quality of silage. Similarly, Arbabi et al. (2010) recorded the increase in NDF content by dried citrus pulp as a silage additive. The pH of silage is significantly affected with the addition of orange 30% concentration. The rapid decrease in pH preserves water soluble carbohydrates and declines deamination and proteolysis by inhibiting prolonged fermentation (Muck, 1993). The decrease in silage pH with the increase in orange pulp observed in this study agrees with the findings of Baba et al. (2020) in an experiment to evaluate the effect of silage quality and productivity with citrus pulp. Likewise, Arbabi et al. (2008) examined the effects of citrus pulp, sugar beet pulp and wheat straw as silage and reported the decrease in pH with the increased levels of citrus pulp.

Table 4.2. Chemical composition of alfalfa, orange, and mandarin silages

Unit	Sample	Treatment	ADM	DM	XA	CP	NDF	ADF	pH
1	ALF	Control	25.94±0.50	94.59±0.27*	10.67±0.16	26.57±0.05*	37.00±0.72	31.75±0.07	4.95±0.14
2	ALFO 20	ALF80%+O20%	27.94±0.20	93.01±0.25	13.69±0.54	23.55±0.70	38.39±0.15	39.96±1.35*	5.06±0.03
3	ALFO 25	ALF75%+O25%	26.65±1.09	92.67±0.43	13.39±0.35	19.77±0.14	36.79±0.96	32.23±0.16	4.96±0.01
4	ALFO 30	ALF70%+O30%	27.99±0.26	92.20±0.16	14.40±0.06*	22.00±0.22	39.11±1.15	25.54±8.97	4.12±0.01
5	ALFM 20	ALF80%+M20%	28.78±0.08	91.42±0.90	13.40±0.24	23.92±0.18	36.46±1.14	32.85±0.29	5.17±0.06
6	ALFM 25	ALF75%+M25%	28.85±0.01	93.12±0.29	12.48±0.15	20.51±0.39	39.96±1.35*	31.61±0.48	5.70±0.10*
7	ALFM 30	ALF70%+M30%	29.37±0.54*	92.60±0.15	12.69±0.22	21.69±0.20	38.64±1.86	29.76±0.26	5.36±0.15

ADM = air dry matter, DM = dry matter, XA = ash content, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber

At $P < 0.05$ all the tested samples were not statistically different from each other.

* Shows the sample that ranked highest for each.

4.3 *in vitro* True Digestibility of Alfalfa, Orange, And Mandarin by Using Ankom Daisy Incubator

The *in vitro* true digestibility trials of the individual samples (ALF-hay, Orange & Mandarin) are listed in table 4.3. The results showed that alfalfa-hay had higher (77.95±0.99) *in vitro* true digestibility (as fed basis) followed by mandarin (77.23±3.24) and orange (76.70±3.86). Likewise, a noticeable increase (76.08±1.08) was observed in ALF-Hay *in vitro* true digestibility (DM basis) compared to Orange (74.73±4.18) and mandarin (75.30±3.52). The neutral detergent fiber digestibility (NDFD) was noted higher (31.82±3.07) for ALF-Hay whereas lowest (24.08±10.81) for Mandarin.

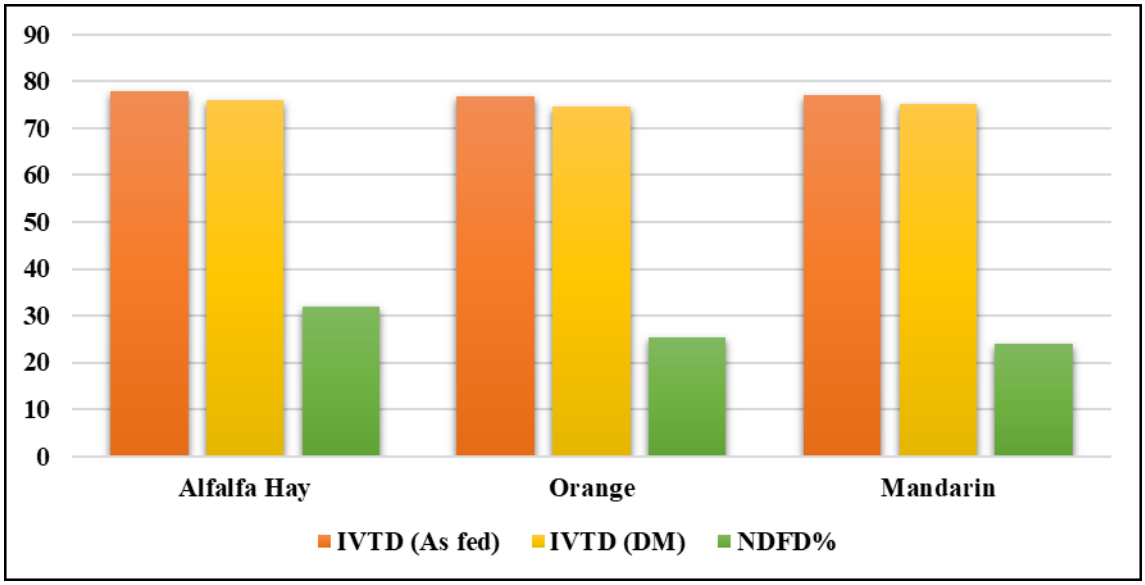

Figure 4.3. *In vitro* true digestibility of alfalfa, orange, and mandarin by using Ankom Daisy^{II} incubator

The feeding value of feeds are associated with both their nutrients composition and their digestibility. The digestibility of feed mixtures may differ from their individual digestibility (Niderkorn and Baumont, 2009). However, feed supply and quality are one of the fundamental problems in ruminant nutrition. These findings are comparable to the observations of Gomes et al. (2017) reported that addition of citrus pulp improves the chemical composition of the silage and it's *in vitro* true digestibility. Likewise, the current study showed comparatively higher *in vitro* true DM digestibility of orange and mandarin than reported by Kour et al. (2014) for variable levels of Kinnow mandarin waste

(IVTDDM 50-55%). The *in vitro* NDF digestibility did not differ significantly across alfalfa hay samples whereas NDF digestibility was clearly affected in orange and mandarin samples. It is because that the fiber in orange and mandarin is highly fermentable that might lead one to expect the digestion of NDF should have increased. The results obtained agreed with those reported by Villarreal et al. (2005) who observed 77.7% *in vitro* digestibility (as fed) of alfalfa silage. According to Alnaimy et al. (2017), citrus pulp has high easily fermentable carbohydrates and some rich energy nutrients for ruminal microbes that enhanced the NDF digestibility coefficients.

Table 4.3. *In vitro* true digestibility of alfalfa, mandarin, and orange by using Ankom Daisy^{II} incubator

Unit	Sample	IVTD (As fed)	IVTD (DM)	NDFD%
1	ALF-Hay	77.95±0.99*	76.08±1.08*	31.82±3.07*
2	Orange	76.70±3.86	74.73±4.18	25.26±12.37
3	Mandarin	77.23±3.24	75.30±3.52	24.08±10.81

IVTD (As fed) = *In vitro* digestibility (as fed), IVTD (DM) = *In vitro* dry matter digestibility, NDFD% = (Nutrient detergent fiber digestibility)

At $P < 0.05$ all the tested samples were not statistically different from each other.

* Shows the sample that ranked highest for each.

4.4 *in vitro* True Digestibility of Alfalfa, Mandarin, and Orange Silages by Using Ankom^{ii} Daisy Incubator

The data related to *in vitro* true digestibility parameters are shown in table 4.4. There were highly statistically significant differences in the digestibility of silages. The IVTD (*in vitro* true digestibility) (as fed basis) showed highly significance difference. The highest value (84.61±0.9) of IVTD (*in vitro* true digestibility) (as fed basis) was found for ALFO30 (Alfalfa hay-wilted 0.70 + orange pulp 0.30) which was statistically at par with ALFO25 (82.16±0.59) and ALFO20 (82.85±0.28), while the lowest (76.60±1.09) were found for control. Similarly, ALFO30 resulted for maximum (83.38±0.97) IVTD (DM basis) followed by ALFO20 (81.18±0.30) and control for the minimum (75.24±1.15) value respectively. The control showed the less amount (33.22±3.12) of neutral detergent

fiber digestibility (NDFD) whereas, ALFO30 showed comparatively higher (57.08±2.51) neutral detergent fiber digestibility.

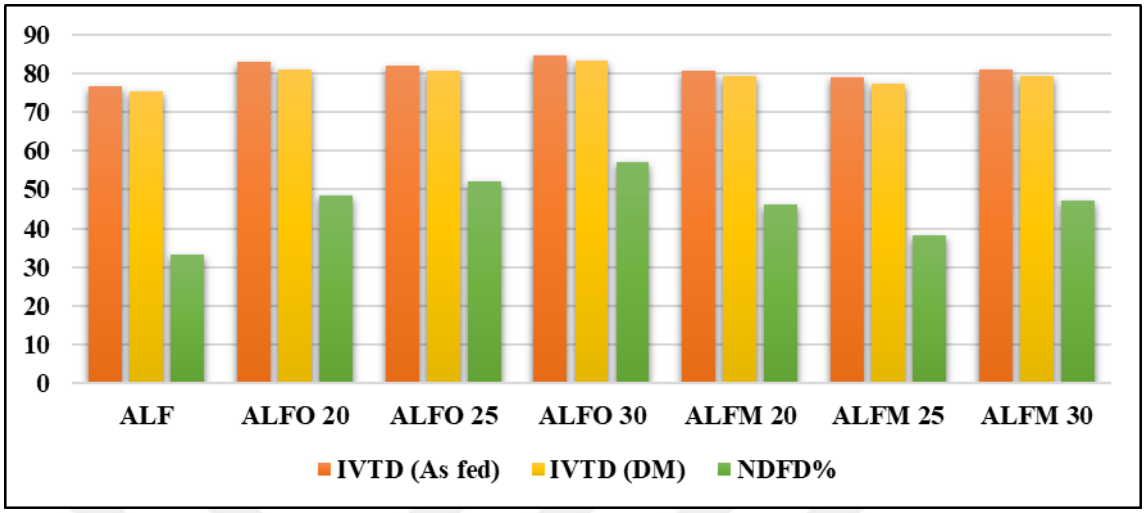

Figure 4.4. *In vitro* true digestibility of alfalfa, mandarin, and orange silages by using Ankom^{II} Daisy incubator

In mixed feeds, replacing starchy diets with those rich in easily fermentable cell walls, such as citrus pulp, prevents, at least in part, the negative impact of high concentrate levels on forage digestibility (Barrios-Urdanetat et al. 2003). In this research, the orange and mandarin supplementation increased the *in vitro* true digestibility of alfalfa silages. Results of this study demonstrated that different concentration of orange and mandarin supplemented in alfalfa silage significantly differs the *in vitro* digestibility of NDF, DM and as a feed source. According to previous *in vitro* findings from Besharati et al. (2017) revealed that ensiling alfalfa with orange pulp and molasses can enhance silage quality, gas production and *in vitro* DM digestibility. Our results are consistent with the findings of García-Rodríguez et al. (2019) that showed the highest rate of fermentation and high volume of gas production and indicated that citrus was the more degradable and digestible byproduct containing less NDF and ADF, and thus were fermented at a faster rate and more extensively than other byproducts. Barrios-Urdanetat et al. (2003) stated that increasing citrus proportion in silage directly increase the digestibility of neutral-detergent fiber. The findings of Rofiq and Gorgulu, (2014) are comparative close to our findings and reported that orange peel essential oils promote stronger microbial activity for fermentation and affects the *in vitro* digestion of dairy total mixed ration (TMR).

Alnaimy et al. (2017) reported an improvement in NDF and ADF digestibility on supplementation of citrus BPF when substituted for starchy feeds.

Table 4.4. *In vitro* true digestibility of alfalfa, mandarin, and orange silages by using Ankom Daisy^{II} incubator

Unit	Sample	Treatment	IVTD (As fed)	IVTD (DM)	NDFD%
1	ALF	Control	76.60±1.09^{d}	75.24±1.15^{d}	33.22±3.12^{d}
2	ALFO 20	ALF80%+O20%	82.85±0.28^{ab}	81.18±0.30^{ab}	48.67±0.84^{ab}
3	ALFO 25	ALF75%+O25%	82.16±0.59^{ab}	80.84±0.63^{ab}	52.17±1.58^{ab}
4	ALFO 30	ALF70%+O30%	84.61±0.9^{a}	83.38±0.97^{a}	57.08±2.51^{a}
5	ALFM 20	ALF80%+M20%	80.79±0.67^{bc}	79.35±0.72^{bc}	46.34±1.89^{b}
6	ALFM 25	ALF75%+M25%	78.90±0.61^{c}	77.24±0.66^{c}	38.27±1.79^{c}
7	ALFM 30	ALF70%+M30%	80.86±0.29^{b}	79.24±0.32^{b}	47.04±0.81^{ab}

ALF = Wilted Alfalfa, ALFO = Alfalfa orange, ALFM = Alfalfa mandarin, IVTD (as fed) = *In vitro* digestibility, IVTD(DM) = *In vitro* dry matter digestibility

Note: a-d superscripts represent points of statistical significance at P<0.05.

CHAPTER V

CONCLUSION

Agro-industrial by-products wastes that either come from the vegetable processing industry or agricultural crops and their disposal poses an environmental risk as they contain potential pollutants. Incorporating it in ruminant diets is one of their most potential alternative uses. The main aims of this research study were to determine the effects of supplementing alfalfa silage with orange and mandarin pulp in *in vitro* true digestibility. This study also aims to incorporate the orange and mandarin by-products in animal feeds to provide some essential nutrients and improve digestion, positively affecting the health, development, and productivity.

Alfalfa hay, orange, mandarin, and their silages were used to determine their chemical composition, *in vitro* true digestibility, DM, and NDF digestibility. Supplementation of orange and mandarin pulp increased the DM, Ash, CP, and NDF content of alfalfa silage and decreased the pH and ADF content by improving the fermentation process. The results revealed that addition of orange and mandarin to alfalfa silages increased the *in vitro* true digestibility. There was significant difference in all the treatments effecting IVTB (as fed), IVTD (DM) and NDFD% of silage. All the different ratios of orange and mandarin supplements affected the chemical composition and *in vitro* digestibility. Among the treated samples, treatment ALFO30 (Alfalfa hay-wilted 0.70 + orange pulp 0.30) showed promising results for enhancing the chemical composition of silage and improving the *in vitro* true digestibility, DM and NDF digestibility.

It is concluded that the addition of orange pulp (30%) increases the chemical composition of silage, benefits the fermentation process, and enhances the *in vitro* true digestibility of silage. This study suggests the supplementation of 30% orange pulp to alfalfa silage as it improves the nutritive value and digestibility of silage. This study will enable animal nutritionists to understand the importance of citrus based by-products in ruminant's feed.

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