A.M, GARBA, 2021

MASTER THESIS

NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

T.R.
NİĞDE ÖMER HALİSDEMİR UNIVERSITY
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DEPARTMENT OF ANIMAL PRODUCTION AND TECHNOLOGIES

EVALUATION OF NUTRITIONAL COMPOSITION AND IN VITRO DIGESTIBILITY OF APPLE POMACE OBTAINED FROM APPLE CULTIVARS (STARKING, GOLDEN DELICIOUS, AND GRANNY SMITH) GROWN IN NİĞDE

ABDULHAMID MUHAMMAD GARBA

SEPTEMBER, 2021

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ABDULHAMID MUHAMMAD GARBA

Master Thesis

Supervisor

Asst. Prof. Dr. Sema YAMAN FIRINCIOĞLU

September, 2021

The study titled "**Evaluation of nutritional composition and in – vitro digestibility of apple pomace obtained from apple cultivars (starking, golden delicious, and granny smith) grown In Niğde**" and presented by **Abdulhamid Muhammad GARBA** 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 09/02/2021 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 …./…./20.... and numbered ……

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Prof. Dr. Murat BARUT
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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.

Abdulhamid Muhammad GARBA

SUMMARY

EVALUATION OF NUTRITIONAL COMPOSITION AND IN VITRO DIGESTIBILITY OF APPLE POMACE OBTAINED FROM APPLE CULTIVARS (STARKING, GOLDEN DELICIOUS, AND GRANNY SMITH) GROWN IN NİĞDE

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September 2021, 52 pages

The study aimed to evaluate the nutritional composition and in – vitro true digestibility of apple pomaces obtained from three apple (*Malus domestica Borkh*.) cultivars - Golden delicious, Starking and Granny Smith. The apple pomace samples were not significantly different in terms of the parameters measured (P>0.05) for chemical analysis and total phenolics content (TPC) except for in-vitro true digestibility. However, Starking pomace sample (SPS) had higher ADM 27.24%, Golden delicious pomace sample (GdPS) had higher DM 95.3% and Ash 2.00% values while Granny smith pomace sample (GsPS) had the higher NDF value 29.80%, CP 5.09 and ADF 25.30% respectively. GsPS have the highest TPC 112.4 mg GAE/100g compared to SPS 103 mg GAE/100g and GdPS 75.8 mg GAE/100g. SPS had higher IVTD (as fed) 92.36% and IVTD (DM) 92.23%. GsPS had significantly different ($p < 0.05$) NDFD from GdPS and SPS. Based on the result apple pomace can serve as an important component of ruminant ration.

Keywords: Apple pomace, golden delicious, starking, granny smith, nutritive value, in vitro true digestibility

ÖZET

NİĞDE'DE YETİŞEN ELMA ÇEŞİTLERİNDEN (STARKİNG, GOLDEN DELİCİOUS VE GRANNY SMİTH) ELDE EDİLEN ELMA POSASININ BESİN BİLEŞİMİ VE İN VİTRO SİNDİRİLEBİLİRLİĞİNİN DEĞERLENDİRİLMESİ

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Eylül 2021, 52 sayfa

Çalışma, üç elma (*Malus domestica Borkh*.) çeşidinden elde edilen elma posasının besin bileşimini ve in-vitro gerçek sindirilebilirliğini değerlendirmeyi amaçlamıştır. Elma çeşitlerinin posaları, kimyasal analizlerde ölçülen parametreler bakımından önemli derecede farklı bulunmamıştır (P>0.05). Ancak, sayısal olarak, Starking posası (SPS) daha yüksek ADM %27.24, Golden delicious posası (GdPS) daha yüksek DM %95.30 ve kül %2.00 içerirken, Granny smith posası (GsPS) daha yüksek NDF değeri %29.80, ham protein %5.09 ve ADF %25.30 içermiştir. Elma posası örnekleri toplam fenolik içeriği bakımından önemli derecede farklı bulunmamışlardır (P>0.05). GsPS, SPS103 mg GAE/100g ve GdPS 75.8 mg GAE/100g göre en yüksek TPC 112.4 mg GAE/100 g olarak bulunmuştur. Elma posası örnekleri in-vitro gerçek sindirilebilirlik açısından önemli derecede farklı olmuştur (P<0.05). SPS en yüksek havada kuru IVTD (as fed) %92.36 ve kuru madde in-vitro gerçek sindirilebilirliği IVTD (DM) %92.23 sahip olmuştur. GsPS, GdPS ve SPS'den önemli ölçüde farklı (P < 0.05) NDFD'ye sahipti.. Bu sonuç, elma posasının geviş getirenlerin geleneksel yem rasyonlarında yer alması veya en azından değiştirmek için kullanılabileceğini göstermiştir.

Anahtar Sözcükler: Elma posası, golden delicious, starking, granny smith, besin değeri, in vitro gerçek sindirilebilirlik

ACKNOWLEDGMENTS

All praise is to Allah the lord of the world who spared my life with full health and ability to reach the successful completion of this thesis/research. May Allah's peace and blessing be upon our noble prophet Muhammad (S.A.W). My profound gratitude's goes to my amiable and amicable supervisor Asst. Prof. Dr. Sema Yaman Fırıncıoğlu for her support and guidance throughout the conduct of the thesis, May Allah in his infinite mercy bless her abundantly with full health and prosperity.

I will not forget my Family Jamilu, Umar, Muftahu, Hamidan, Anas, Aisha, Fatimah, Abubakar, Junaidu, Rafi'a, Maimuna, Najib and Kashif, my wife Zainab Ishaq Sa'id, and my daughter Fateemah for their endless love and support towards my educational commitments, lots of love.

Finally, I am grateful for the knowledge and experiences gained from the entire staff of the department as well as financial support of Ayhan Sahenk Foundation provided to me during this MSc program.

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SYMBOLS AND ABBREVIATIONS

Symbols	Meaning
%	Percentage
°	Degrees
°C	Degrees celsius
cm	Centimeter
g	Gram
g/L	gram/liter
GAE	Gallic acid equivalent
h	Hour
Kg	Kilogram
Kg/m^3	Kilogram per cubic meter
L	Liter
min	Minute
mL	Milliliter
mm	Millimeter
sec	Second
t	Tonnes
w/w	Weight by weight

Abbreviations	Meaning
ADF	Acid Detergent Fiber
ADM	Air Dry Matter
ANOVA	Analysis of Variance
CF	Crude Fiber
CP	Crude Protein
DM	Dry Matter
EE	Ether Extract
IVNDF	In-Vitro Neutral Detergent Fiber
IVTD	In-Vitro True Digestibility

NDF	Neutral Detergent Fiber
NDFD	Neutral Detergent Fiber Disappearance/ Digestibility
TMR	Total Mixed Ration
XA	Ash Content

CHAPTER I

INTRODUCTION

1.1 Background of Study

The apple *Malus* x *domestica* Borkh is a widely distributed, temperate zone fruit crop that has been cultivated for millennia. The apple is a member of *Maleae* tribe, *Malinae* subtribe, *Rosaceae* family, *Amygdaloideae* subfamily and *Malus* genus (Potter *et al.,* 2007). The *Rosaceae* family is distributed worldwide and includes a range of economically important fruit crop species such as the pome fruit species (for example: apple, pear and quince), the stone fruit species (for example: sweet and sour cherry, plum, prune, apricot and peach) and the berry fruit species (for example: strawberry, blackberry and raspberry). The genus *Malus* consists of six sections with 27 primary species (Forsline, 2003). Most of the species belonging to the *Malus* genus are cross-compatible, hence natural and artificial hybridisation techniques have resulted in numerous interspecific hybrids and secondary species.

Apples were domesticated in the Tien Shan Mountains of Central Asia between 4000 and 10000 years ago. The *Malus* genus is thought to have originated in the southeast of the People's Republic of China, and the species of the genus have since spread around the world. *Malus sieversii* (Ledeb.) M. Roem., the cultivated apple's assumed main parent, grew wild in Central Asian woodlands from Tajikistan to the Western People's Republic of China (Luby, 2003; Hancock *et al*., 2008). Other species of the genus (*Malus)* had donated to the cultivated apple's genome, according to genomic research conducted in the previous century. These were mainly *Malus sylvestris* (L.) Mill. distributed from Western Asia to Europe, and *Malus orientalis* Uglitzk which grew in the forests of the Caucasus region (Hancock *et al.,* 2008).

The cultivated apple is one of the *Malus* genus. Tamed apple binomial denomination of reflects its interspecific origin and replaces the former name *M. pumila* Mill., as well as other names like *Pyrus malus* L. *Malus* × *domestica* is allopolyploid (2n = 2x = 34), gametophytic incompatible, mostly self-unfruitful and requiring pollination. Most of the cultivars are diploid; however, a number of tri- and tetraploid cultivars also exist.

Apples have many uses, they can be consumed immediately from tree and can be kept for long in adjustable circumstances. They are common in pies, pastries and cakes, and they can be made into slices, sauce, and, juice. The juice can be converted into fresh juice, cider, wine, and vinegar. The floral display and lovely foliage of Crab apples with attractive qualities are also well understood. Only a small number of significant varieties currently dominate global apple production (O'Rourke, 2003) despite the fact that there are around 6000 based regionally important cultivars and land races around the world. The most popular cultivars include Starking, Granny, Delicious, Golden, fuji & Gala. These cultivars responsible for more than 60% of global production. 'Cripps Pink,' 'Honeycrisp,' 'Scifresh,' 'Delblush,' 'Civni,' 'Corail,' and 'Ariane' are some of the newer kinds.

The apple fruit is among the humanity's first fruits, and it is extensively farmed in colder area (Musacchi and Serra, 2018). Apples are the 4th most known fruit in the planet, behind bananas, oranges, and grapes as reported by (Forsline, 2010). According to (Musacchi and Serra, 2018), global per capita apple consumption had topped 9 kg in 2013 and had been increasing over time. According to a recent (FAO, 2018) report, in the last two decades, global apple cultivation has surged by 48 %, increasing to 83.1 million metric tons by 2017. 65.4 % of world output is been provided by Asia, and 41.4 million mt by China as world's number 1 apple producer. Other major producers are USA, Poland, and Turkey, which contribute for 6.2 percent 2.9 percent and 3.6 percent of total global output, accordingly (figure 1.1). Despite the fact that global apple production is increasing, global apple consumption is basically unchanged. About 75 percent of the total production is been consumed remainder 25% being processed into various items such as juice, wine, jellies etc (Shashi *et al.*, 2008; Shalini and Gupta, 2010). According to reports, apple juice remain the number 1 apple product, dominating 65 percent among all processed apples (Kammerer *et al.*, 2014). During juice production, around 75% of the apple's fresh mass is intended to be generating as juice, while the remaining collected as agro - industrial waste, or as apple pomace (Vendruscolo *et al.*, 2008).

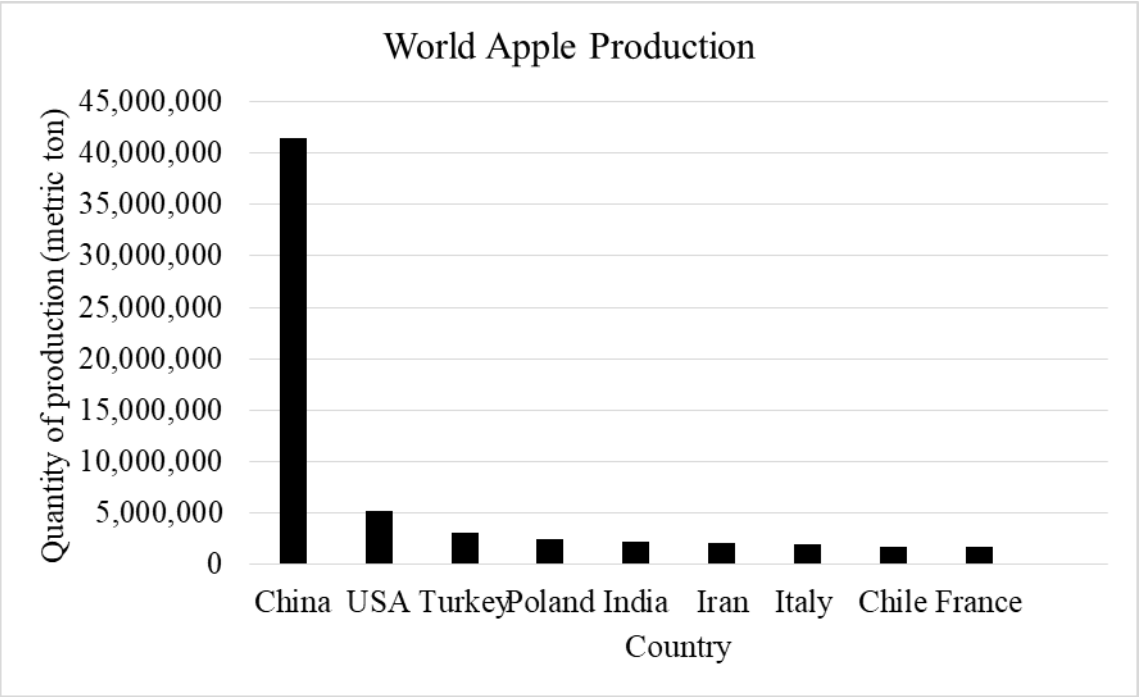

Figure 1.1. Top ten (10) world apple producing countries (FAO, 2018)

According to (Kammerer *et al.*, 2014) every year, many million metric tons of pomace are produced due to global mass manufacturing of juice. Table 1 shows the estimated volume of AP production in various nations, which is expected to grow in the near future. This by-product, on the other hand, has a low recovery rate. According to (FAO, 2003), 71 million tonnes of apples were consumed directly in 2017, there were 5.8 million tonnes processed. 7 million tonnes thrown away, but just 0.66 million tonnes were used as animal feed. The most common method of dumping this by-product is straight into the soil in a landfill, and this is connected to key environmental issues such as pollution (Perussello *et al.*, 2017; Shashi *et al.*, 2008; Shalini and Gupta 2010; Singha and Muthukumarappan, 2018). It is owing to the much water % greater than 70% and high biologically degradable organic load of apple pomace. The former increases the vulnerability of microbial activities, leading to an unexpected fermenting, whereas the latter contributes public health problems and pollution (Shashi *et al.*, 2008). According to studies, apple juice companies should utilize professional waste disposal to minimize environmental issues, which may need considerable financial expenses (Masoodi and Shah, 1994). As a result, research into safe and effective treatments for apple pomace, as well as its application in food and ruminant feeds, is required. Due to the high capacity of apple pomace formed during juice generation and processing, commercial

applications of pomace can have a significant economic impact (Shalini Gupta, 2010). Only a few breakthroughs have been made in the recycling of agro-food processing by-product waste into economically usable goods such as biofuels, nutrients, and multifunctional substances, according to (Laufenberg *et al.,* 2003) in an effort to improve the recovery rate of apple pomace, many uses have recently been studied.

Table 1.1. Production figures for apple pomace in various nations

Nation	**Quantity (Metric Tons)**	**Ref.**
Brazil	800,000	(Vendruscolo *et al.,* 2008)
China	1,000,000	(Wang *et al.,* 2010)
Germany	250,000	(Endreß, 200)
India	1,000,000	(Shalini and Gupta, 2010)
Iran	97,000	(Pirmohammadi *et al.*, 2006)
Japan	160,000	(Takahashi and Mori, 2006)
United States	27,000	(Roberts *et al.,* 2004)

1.2 Importance of Apple Pomace

Apple pomace (AP) as an industrial by-product is high in carbohydrates and minerals but low in protein. Direct burning, fuel (ethanol production), methane production, gasification, pomace jam, sauce, confectioneries, citric acid generation, pectin extraction, fibre, and animal ration are just a few of the applications for apple pomace (Shalini *et al.,* 2010). Because apple pomace has a high moisture content and is high in fermentable sugars, ensiling or dehydration is used to keep it fresh for longer (Shalini *et al.,* 2010).

After ensiling or drying, apple pomace has traditionally been used as an animal feed (Besharati and Taghizadeh, 2008; Kafilzadeh *et al.,* 2008). Ewes' weight gain and lambing performance were found to be negatively affected by a diet consisting predominantly of urea-supplemented AP (Rumsey and Lindahl, 1982), as had previously been documented with cows (Heuzé *et al.,* 2020). Furthermore, as previously demonstrated with cows, included straw in the diet reduced the negative consequences (Rumsey and Lindahl, 1982). When AP was mixed thoroughly at varying level with wheat barn, chopped alfalfa, and milled rice barn, ensiled and then fed to Holstein milk cows according to (Toyokawa *et al.,* 1984) revealed higher milk and milk fat, as well as

lower feeding cost. Another study found that apple pomace when ensiled can successfully replace about 30% of a diet's calories without affecting milk output or composition (Ghoreishi *et al.*, 2007). A study found that pomace from puree making was more digestible than apple pomace from juice production (Kafilzadeh *et al.*, 2008).

1.3 Aim of the Research

Agricultural industrial waste products have sparked greater attention due to a variety of issues including pollution control and laws, rising waste disposal costs, and shifting perceptions of the importance of bye-product feedstuffs as cost-effective feed substitutes. Understanding the nutritive value and a sustainable way of utilizing agricultural industrial waste product would be of advantage to improve animal nutrition and health, animal welfare, lowering of feed production cost, as well as reducing the amounts solid organic wastes in the environment. Although currently underutilized, the potential for using agro-industrial by-products in ruminant animal nutrition is enormous (Tayengawa and Mapiye, 2018). This is due to a lack of finance, skill, technology, and infrastructure in low- and middle-income countries (Tayengawa and Mapiye, 2018).

The aim of this study is to evaluate the nutritional composition and, *in vitro* true digestibilty of apple pomace obtained from three varieties of Apple - Starking, Golden delicious and Granny smith grown in Niğde.

CHAPTER II

LITERATURE REVIEW

2.1 Apple Production in Turkey

Pear, medlar together with quince, loquat, and apple belongs to family *Rosaaceae* and the subfamily *Pomoideae* (Özbek, 1978). Despite the domestic apple originated in Caucasia, Turkistan, Anatolia, and Europe, it is currently grown in both the Northern and Southern Hemispheres in continental climates. Apple was domesticated for a long time and is one of the most popular fruits for human consumption. Apple trees are among the successful plants in the universe, with over 7500 registered rootstocks and cultivars (Mukhtar *et al.,* 2010).

Turkey is one of the world's leading apple producers, with an estimated production of 3,128,000 tons in 2017. (FAO, 2018). The noteworthy apple cultivars grown in Turkey are Golden delicious, Starking, Amasya, and Granny Smith. In recently established apple orchards, new varieties are harvested from June - October. Mondial Gala and Galaxy Gala cultivars have provide additional gain by preventing crop build-up in the late season and enhancing output of mid-season apple varieties. Early types such as Stark earliest, Jerseymac, Vista Bella, Anna, and others are produce in Turkey's subtropical districts of Hatay, Adana, Mersin, and Antalya. The (table 2.1) below shows the quantities of apple produced in Turkey by major varieties from 2015 to 2019 by (FAO, 2018).

Table 2.1. Cultivars of apple in Turkey and quantity of productions (Ton)

Provinces	**2015**	**2016**	**2017**	**2018**	**2019**
	Quantity (Tons)	**Quantity (Tons)**	**Quantity (Tons)**	**Quantity (Tons)**	**Quantity (Tons)**
Granny Smith	121,674	134,448	140,000	150,529	152,680
Starking	1,002,500	1,140,060	1,215,157	1,299,390	1,323,104
Amasya	230,285	232,120	192,756	217,433	219,299
Golden	680,500	750,650	798,137	864,247	881,897
Others	534,800	668,550	686,114	1,094,361	1,041,772
Total	2,569,759	2,925,828	3,032,164	3,625,960	3,618,752

2.2 Origin of the Apple Cultivars Used

2.2.1 Starking/red delicious

Jesse Hiatt discovered the first Starking apple as a fortuitous sprout near Peru, Iowa, in 1881. In 1894, Stark Bros (SB) Nurseries in Louisiana, Missouri, purchased the authority to Starking and extensively propagate it. Growers and nurserymen have propagated over 100 Red Delicious strains to date. Starking is a sweet, mellow apple that should be eaten rather than cooked. Red delicious trees are productive and tolerate a variety of growth situations, according to the (USDA, 2003).

2.2.2 Golden delicious

Golden Delicious is a type of apple that developed in West Virginia circa 1900. It is unrelated to the Red Delicious apple save for the fact that it was similarly bought, propagate, and named by SB Nurseries. It's worth noting that this 2 important apples aren't the outcome of commercial fruit breeding. The flavor of Golden is sweet and soft, and they preserve quite good. Golden Delicious has spawned a slew of offspring. Several current cultivars, including Jonagold, Spigold, and Gala, are descended from the Golden Delicious Mutsu (USDA, 2003).

2.2.3 Granny Smith

The Granny Smith apple, the world's third most popular, has been around since the 1860s. Granny Smith was named after an accidental seedling found in Sydney, Australia at Marie Smith's back yard. Granny Smith apple is mostly grown commercially on the West Coast of the United States and requires a long growing season. It's a hard, green, juicy, tart apple that's great for apple pie and helps juice production by adding acidity. Jonathan was a seedling of Esopus Spitzenburg seedling. Even though it is no longer a popular cultivar, The Esopus Spitzenburg apple originated in Esopus, New York, in the 18th century, and is reputed to have been Thomas Jefferson's selected apple produced at Monticello (USDA, 2003).

Gala, Jonagold, and Fuji are some of the newer cultivars that have come from breeding initiatives. Gala is a breed between Kidds Orange and Golden Delicious that developed in New Zealand. As previously mentioned, Jonagold is a hybrid between Golden Delicious and Jonathan that developed in New York State. Fuji is a mix of Delicious and Ralls Janet that originated in Japan. Several red variants of these cultivars have been chosen and are now being produced and sold to the general public by the (USDA, 2003). Picture of some important apple cultivars is shown in (Figure 2.1) below.

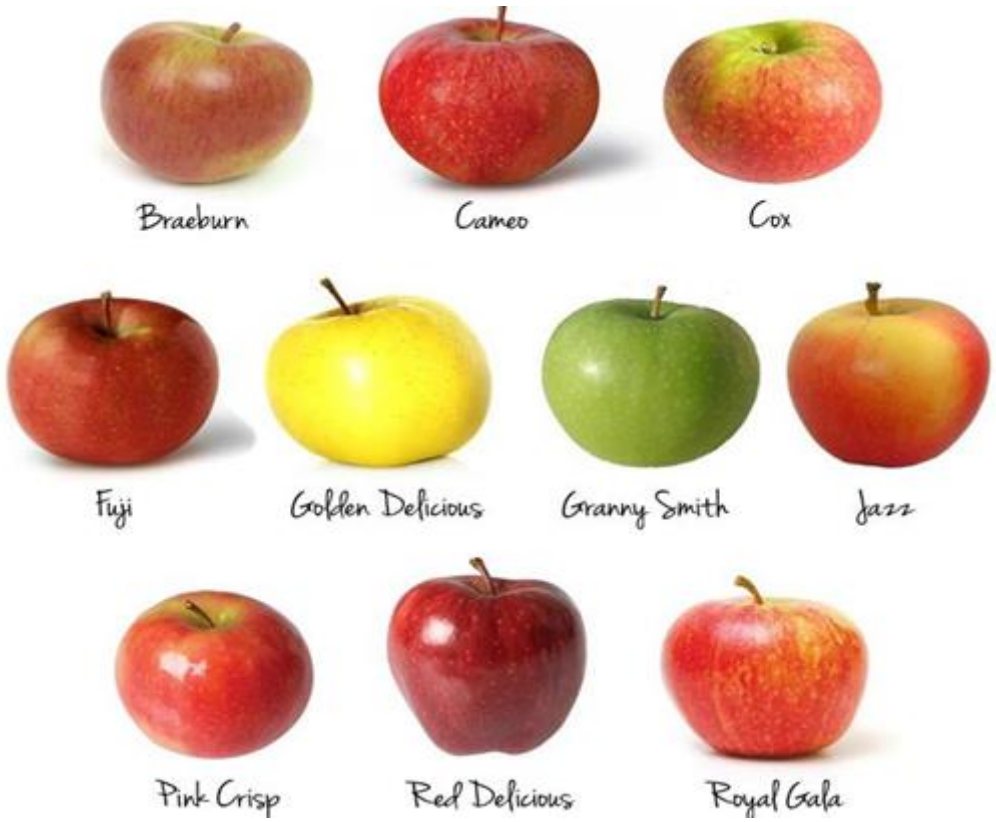

Figure 2.1. Important apple varieties

2.3 Apple Pomace as an Agro – Industrial by-Product

Apple pomace is the solid residue left behind after processing apples for cyder, and juicen (*Malus domestica Borkh*) (Givens and Barber, 1987; Kafilzadeh *et al.*, 2008). While the apple season, culled, fallen, and injured apples (damaged, hurt while plucking, and unsuited for loading) were many, and are occasionally used to feed cattle (NDDB, 2012). Almost 75 % of the total apple crop is consumed fresh, and the rest are damaged or having low quality, and so is not marketed. Damaged apples are

turned into value-added goods ((Dhillon *et al.,* 2011). Using a traditional approach, 71-74 percent of an apple's fresh weight is extracted as juice, leaving the remaining 25-30 percent as apple pomace (Fig. 2.2). (Aghsaghali and Sis, 2008; Wadhwa and Bakshi, 2013).

Peel, flesh, stem, core, seeds, and juice remnants are all found in apple pomace (Sudha *et al.,* 2007). A sample of apple pomace had 54 percent pulp, 34 percent peel, 7% seeds, 4% seed core, and 2% stem, according to the research (Kolodziejczyk *et al.,* 2007). Unpreserved AP has 15-30% DM, while pressed pomace contains 30-40% DM. The density/kgm3 of fresh apple pomace varies between 400 and 1000 reported by (Kennedy *et al.,* 1999). Processing methods and apple maturity influence density and moisture content. The composition and physical features of apple pomace is shown in figure (figure 2.2) below.

There are two forms of waste generated by the apple processing industry. The first category is belt rejection, which refers to fruit that is abandoned at the sorting belt because it is partially bruised or rotten. The AP obtained after juice extraction is the second type. Apple pomace and belt rejection apples are also thrown as rubbish. About 25% of AP and 1.5 % of apples are thrown as processing wastes in large-scale apple processing operations (Shah and Masoodi, 1994). These wastes cause challenges with disposal, contamination, and the loss of valuable biomass and nutrients (Bhushan *et al.,* 2008). Treatment and disposal of such pollutants also come at a high cost (> $10 million USD, according to Bhushan *et al.,* 2008). Wet apple pomace includes a lot of water (70-80%), making it heavy and easily fermentable. It also degrades quickly, making storage and shipping difficult (Bates and Roberts, 2001). It's also quite biodegradable.

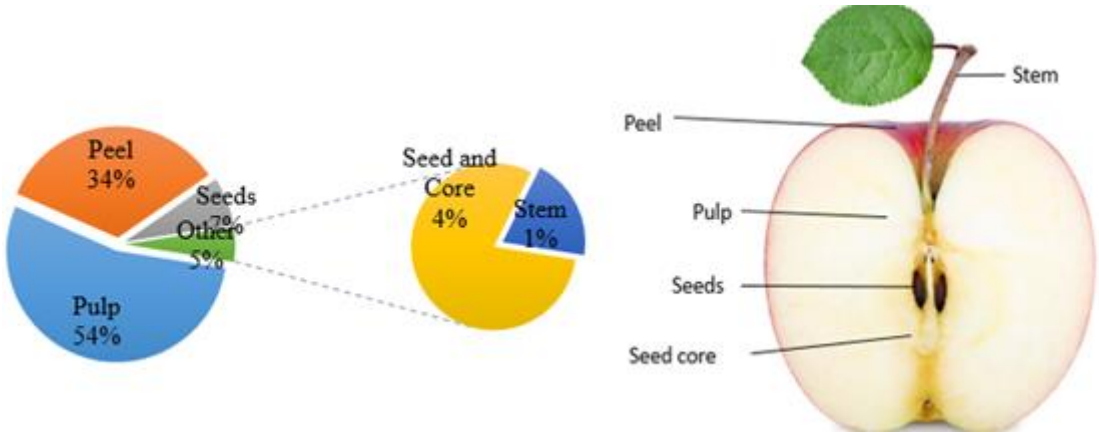

Figure 2.2. Composition of apple pomace

Because apple pomace accounts for nearly a quarter of all apples processed for juice or cider, 1.6 million tonnes of raw pomace from apple were generated globally dated 2017. (FAO, 2018). In France, where apple production was estimated to be at 1.8 million tonnes in 2012, around a 3rd of the plant is converted to juice or other products, leaving about 150 thousand tons of pomace from apple (FNPF, 2016). Cider mills in France, generate approximately 8700 t of the dry matter of apple pomace every year, so also lesser amounts for rejected apples (Roy *et al.,* 2013). Every year, India produces 1 million tons of apple pomace, but only ten thousand tons is used for animal feed as reported by (Shalini *et al.*, 2010). Pomace from apple is found in any cold climate with apple woods. AP is typically employed at the area close to the apple juice and other apple product processing facilities since it degrades easily. Apple pomace is a seasonal commodity that is accessible after the fruit is collected in the fall (Roy *et al.*, 2013). Apple processing and pomace production is shown in (Figure 2.3).

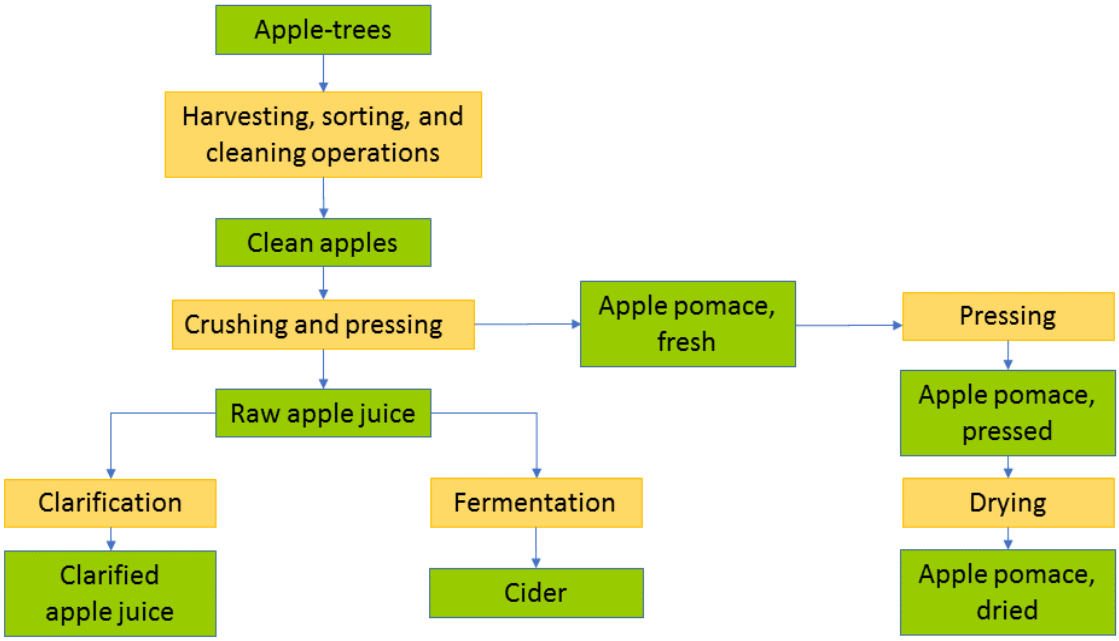

Figure 2.3. Apple processing and apple pomace production

2.4 Collection, Storage and Preservation of Apple Pomace

Because fresh apple pomace has a high moisture content, it degrades quickly. Even if it's going to be fed fresh, it's a good idea to consolidate and sheet it to keep the air out (Hall, 2014). Ensiling apple pomace as if it were grass allows it to be stored for a long time. It is recommended that it be combined with dry materials such as rice hulls or straw to absorb the leachates produced during fermentation and avoid effluent losses (Perry *et al.*, 1995). Air should be eliminated by using a tractor to compact the silo and covering it with a clean, undamaged plastic sheet. 2-3 days exposure before use at a time when feeding begins ensured to avoid air from entering beneath the sheet, (Hall, 2014). The ensiling of AP (EAP) can leads to the ethanol formation, which can be harmful to animals if fed for lengthy periods of time (Crawshaw, 2004).

The water content of fresh apple pomace is high. The first step in drying the pomace is to ensure all the water drained by placing on a slanting surface. After that, the pomace can be squeezed through a filter press and dried in slim strata by warm air aeration, or dried in a sun by way of distributing it in 5-7 cm thick layer on a concrete floor and turning it two to three times a day until the dry matter getting 90%. Dehydration takes only three days in hot locations where the degree of hotness reaches up to 45°C. The

apple pomace is dried and pulverized in a mill before being stored in bags made up plastic (Wadhwa *et al.,* 2013). Figure 2.4 shows how fresh and dried apple pomace looks like.

Figure 2.4. Fresh and dried apple pomace

2.5 Utilization of Apple Pomace

Apple pomace could be used as a waste minimization approach, a high-value product, or a combination of the two. (Endreß, 2000) considers pectin production to be one of the most cost-effective and environmentally friendly ways of utilizing apple pomace, as well as its usage in foods as a gelling agent, thickening, texturiser, emulsifier, and stabiliser (Thakur *et al.,* 1997). Whole apples and their by-products, such as pomace, have a delicious flavor and contain useful components like polyphenols (Schieber *et al.,* 2001). The majority of the phenolic chemicals found in apple pomace are phenolic acids and flavonoids. Flavonoids are the most abundant type of substance in apple pomace, with flavonols being the most abundant subclass, followed by flavonones, flavones, dihydrochalcones, and anthocyanins. These polyphenols in apple / pomace have a number of biological properties, including metal chelation, free radical scavenging (Chien *et al.,* 2007; Sudha *et al.,* 2007), anti-allergic (Akiyama *et al.,* 2005), and anti-arteriosclerotic (Akiyama *et al.,* 2005; Stefania *et al.,* 2007). Furthermore, through solid form fermentation, Citric acid, protein-fortified feeds, fragrance materials, edible mushrooms, and ethanol are all made from apple pomace (Almosnino *et al.,* 1996), natural anti-oxidants, and different enzymes, among other things (Dhillon *et al.,* 2011; Vendruscolo *et al.,* 2008). Soluble dietary fibres from apple pomace give nutritional

benefits by lowering blood cholesterol levels (Kaushal and Joshi, 1995). Apple pomace is abundant in sugars and contains a variety of carbon sources, making it an ideal substrate for a variety of microorganisms in bioprocesses such as the generation of single cell proteins (Vendruscolo *et al.*, 2009). Furthermore, apple pomace includes 5% seeds, which are high in polyphenolics (7.24 g/kg DM), particularly procyanidins and quercetin glycosides (4.46 g/kg DM), which have potent anti-oxidant properties. Apple seeds are high in oil, with linoleic acid (50%) being the most prevalent, followed by palmitic, linolenic, stearic, oleic acids (Lu and Foo 1998). Although it is theoretically possible to process apple pomace for these uses, it is not commercially viable (Kennedy, 1994); As a result, the economics of apple pomace usage are based on its many uses, such as solid state fermentation, an innovative method for recovering ethanol while also creating animal feed (Joshi and Sandhu, 2006). Using apple pomace as livestock feed is one of the most profitable options for apple processing firms to address the current feed shortage.

2.6 Nutritional Composition of Apple Pomace

Core, peel, calyx, seed, stem, and soft edible tissue make up apple pomace, which is a heterogeneous composition. It contains a high amount of fermentable carbohydrate, pectin, crude fiber, and minerals, making it ideal for cattle nutrition. However, the composition changes depending on the apple species, agricultural techniques, fruit ripeness, and extraction process (Pirmohammadi *et al.*, 2006), the most crucial component is the number of times the fruits are pressed (Vendruscolo *et al.*, 2008). Apple pomace has a high water level, insoluble carbohydrates (hemicellulose, cellulose, lignin), sugars (fructose, glucose and sucrose), and high mineral and vitamin C content. It is, however, deficient in protein and important amino acids (Joshi and Attri, 2006). Apple pomace typically comprises 66.4-78.2% moisture and 9.0-22.0 % carbohydrates. Carbohydrate, crude fiber, fermentable sugar, pectin, and mineral content, among other things, determine its worth (Bhushan *et al.*, 2008). The amount of sugar in apple pomace varies depending on the apple variety and the processing method utilized. The amount of water in apple pomace varies depending on the apple cultivar and the stage of ripening (Gullon *et al.*, 2007). The proximate composition of apple pomace is given in Table (2.2). Apple pomace is classified as a high-carbohydrate, low-protein diet (Hang, 1988). It is a poor animal feed because of its low protein and vitamin content, high

sugar content, and seasonal availability. To help it overcome its protein/nitrogen deficiency, salts like urea and ammonia can be added. In ruminant diets including at least some easily available carbohydrates, non-protein nitrogen (NPN) is efficiently utilized. Apple pomace supplemented with natural protein offers a nutritional value for beef cattle that is comparable to grass silage.

The content of apples varies depending on the variety, ripeness, harvest season, and other factors (Grigoras, 2012). Apple pomace is low in protein (between 3 and 11 percent of dry matter) but high in fiber and carbohydrates. Crude fibre makes up 18 to 50% of dry matter, neutral detergent fiber makes up 48 - 75% of dry matter, acid detergent fiber makes up 36-67 % of dry matter, and lignin makes up 16 to 35% of dry matter. Sugar concentration varies as well: apple pomace contains a lot of fructose (14 to 35 percent of dry matter). Saccharose and glucose levels are lower, but still variable, at 1-11 % and 6-13 % of dry matter, respectively (Gullon *et al.*, 2007; Kolodziejczyk *et al.*, 2007). It is important to note that the sugar amount revealed by traditional feed analysis methods is often low (about 6 percent of DM).

Apples have a high level of flavonoids, and a number of other phytochemicals, and their quantity can vary depending on a variety of circumstances, including apple cultivar, harvest and storage, and apple processing. Phytochemical concentrations differ significantly between apple peels and apple flesh. Phloridzin, chlorogenic acid, epicatechin, and quercetin glucosides, among the most well-studied antioxidant chemicals in apple pomace, have all been identified (Lu & Foo, 2000). The antioxidant activity of these phenolics isolated from apple pomace was found to be very high, hinting that apple pomace could have both nutritional and commercial benefits (Lu & Foo, 2000).

Below (table 2.2) show the nutritive value and in – vitro digestibility values of a dried apple pomace reported by (AFZ, 2011; Chapoutot *et al.*, 1990; Gippert *et al.*, 1988; Hindrichsen *et al.*, 2004; Narang *et al.*, 1985; Wolter *et al.*, 1980) *Last updated on 14/09/2016 11:39:03*

Table 2.2. Reported chemical composition of dried apple pomace

Chemical parameter	Unit %DM	Avg	SD	Min	Max	Nb
DM	as fed	91.2	1.3	87.8	94.7	224
CP		8.0	2.3	3.6	11.4	119
CF		36.0	6.8	18.1	49.6	224
NDF		65.1	7.4	48.2	74.6	70
ADF		57.7	8.0	36.7	67.1	70
Lignin		26.8	3.3	16.2	35.0	172
Cell walls, water insoluble		72.8				1
EE		2.8	0.9	1.2	4.7	50
XA		2.1	1.0	0.8	5.0	67
Starch (polarimetry)		8.1	6.2	0.9	18.4	7
Sugar content		5.5	1.8	2.6	13.9	111
Fructose		14.5		14.5	14.5	2
	Unit mg/kg DM	Avg	SD	Min	Max	Nb
Manganese		7		6	9	2
Copper		5		5	5	2
Potassium		1.9	0.7	1.2	3.4	18
Calcium		0.6	0.2	0.2	1.1	25
Zinc		4		3	5	2
Phosphorus		1.4	0.3	1.0	2.0	27
Sodium		2.7	5.3	0.1	15.0	20
Iron		72	19	60	100	4
Ruminant	Unit	Avg	SD	Min	Max	Nb
Digestible Energy ruminants	MJ/kg DM	10.4				
Metabolizable Energy ruminants	MJ/kg DM	8.5				
Organic Matter digestibility, ruminants	%	55.4				
Energy digestibility, ruminants	%	53.7				
N degradability	%	43				1
N digestibility, ruminants	%	50.0				

Avg: average; SD: standard deviation- Max: maximum value Min- minimum value, Nb- number of values (samples) used. * indicate taht an equation was used to get the average value.

2.7 Uses of Apple Pomace in Livestock Feed

Many agricultural by-products have significant potential as animal feedstuffs. As a result, these by-products can largely replace cereals. As a result, the competition for food between people and animals may be reduced. Because they are available for use as livestock feeds at competitive rates, By-product feedstuffs (BPF) in ruminant diets promote growth and lactation, as well as the generation of edible food; as a result, they are becoming increasingly important in the food and fiber system. An alternative promising feeding strategy for economically rearing ruminants is an intense feeding

system based on locally accessible BPF. At times of feed constraint, BPF can also be employed as strategic feeding for ruminants. In ruminant nutrition, pomace from apples can be a useful industrial by-product (Aghsaghali and Sis, 2008). When compared to barley, apple pomace has higher digestible energy and net energy for lactation, resulting in increased synthesis of short chain fatty acids at a lower cost (Khatooni *et al.,* 2014). Despite the fact that it is often used as cow feed (Shalini and Gupta, 2010), It's been successfully fed to sheep (Alibes *et al.,* 1984). Because of the lower protein and nutrient digestibility levels, apple pomace is not an acceptable quality feed for ruminants in practice (Givens and Barber, 1987).

Sugar is plentiful in both apple pomace and whole apples (mostly fructose and glucose), making them an important source of quickly digested energy within rumen (Kolodziejczyk *et al.,* 2007; Gullon *et al.,* 2007). When fed alone, apple pomace must be supplemented with a high protein source and fibrous material such as straw due to its low nitrogen content and consumption level. It's not recommended to utilize it on its own.

Ruminants can eat apple pomace and culled apples (NRC, 1983; NDDB, 2012). Apple picking is a favorite pastime for cattle grazing in apple orchards. Whether fed indoors or out on pasture, pomace from apple can be used up to 30% of the time in a diet of dairy cows. It has no discernible negative impact on total DM intake, milk yield, or milk composition. In a diet for steers that includes a good ryegrass feed, apple pomace substituted half of the maize grain (Ribeiro *et al.,* 2012). The DM intake and digestibility of dried and fresh apple pomace were 1.21 and 1.98 percent of body weight and 43.8 and 57.8 percent, respectively, when fed as sole feed to 175 kg calves supplemented with just minerals (Singhal *et al.,* 1991). Ground and dried apples were utilized in India as a source of energy for crossbred calves, accounting for up to 30% of the concentrate mixture, completely replacing corn (NDDB, 2012). Apple pomace silage should be supplemented with a protein supplement rather than a non-protein supplement when utilized as fibrous feed for beef cows during their gestation period, because non-protein supplemented cows had calves that were lighter, weaker, and had skeletal abnormalities, despite the fact that they were born alive. Furthermore, there is increased calf death (Fontenot *et al.,* 1977).

Fresh pomace from apple was gradually added to a total mixed feed produced as silage and fed to beef cows up to 5 - 20 % on a DM basis, the DM and OM digestibility of With increasing quantities of apple pomace, the TMR declined from 53 to 51 % and from 58 to 55.4 %, respectively (Fang *et al.,* 2016). The needs for gestating ewes were not met with pomace from apple enriched with urea, as it does not match the need for cows. When compared to a control diet, stillbirths were more numerous, birth weights were lower, lamb survival was poor, and structural abnormalities were more common. These unfavorable effects were minimized or avoided when apple pomace was supplemented with a suitable protein source and fiber feed, (Rumsey *et al.,* 1982). Dried or rather ensiled apple pomace added at 20 or 30 percent to a total mixed feed allowed fattening lambs to gain weight quickly and have a larger dressing percentage & carcass (Taasoli *et al.,* 2008). Dietary dry matter consumption was higher in sheep when pomace of apple was enriched with a good quality N, such as alfalfa instead of urea (Alibes *et al.,* 1984). Apple pomace was discovered to be a suitable feed for pigs in early tests. When feeding apple pomace to pigs, the volume of pomace as well as the length of the feeding time must be taken into account (Fang *et al.,* 2016). Apple pomace was fermented with fungus, which resulted in a 36 % increase in crude protein content. Apple pomace that has been enhanced was added to the diets of developing pigs at a rate of 5% and increased ratios of feed conversion (Ajila *et al.,* 2015).

The literature on the use of apple pomace as a poultry feed is limited because of its low protein. Broilers can only get their energy from fresh apple pomace. Apple pomace with a fiber concentration greater than 10% resulted in moist litter and reduced feed efficiency.

The effect of apple pomace on rabbits was studied, and it was discovered that increasing dietary fibre content with apple pomace increases ratio of muscle fatty acids in smaller rabbits (Carrilho *et al.,* 2009). Dried apple pomace should only be used as a safe source of soluble (digestible) fiber in rabbit diets, and it should be supplemented with other components.

Apple pomace is a horse's favorite food. It's frequently used as a stand-alone horse treat or in a blend with oats as reported by (Worth, 2010). Apple slices are also frequently utilized to make horse treats.

The substrate for solid state fermentation and the generation of protein-enriched feed for tilapia fry was apple pomace. The fry grew 44 percent faster on the experimental diet than on the control diet (Vendruscolo *et al.,* 2009).

2.8 Potential Problems Associated with the Use of Apple Pomace as Feed

In apple pomace silage, ethanol is the most common fermentation end product, which has been observed to contain up to 17 % DM. As a result, it was suggested that pomace be fed to cattle in small amounts or for brief periods of time (Alibes *et al.,* 1984). A silage made out of intact, crushed apples had an ethanol content of 22-29 percent of DM, which could have been caused by yeast alcoholic fermentation. It was advised that up to 30% of the DM be replaced with a dry and fibrous material such as straw because the pH could not be lowered and the silage could not attain a stable state (Rodrigues *et al.,* 2008).

The peel of culled apples and apple pomace includes a substantial amount of lipophilic pesticide residues (Amvrazi, 2012). Ingested pesticides can be retained at low levels in adipose tissues and milk (Chase *et al.,* 1987). Pesticide remnants have been discovered in various examinations recently (Lozowicka *et al.,* 2015; Pogăcean *et al.,* 2014), hundreds of pesticides have been approved for use in production of apple all around the globe (Drogué *et al.,* 2012). Although the majority of residues were determined to be within the local regulation's Maximum Residue Levels, some were discovered to be above this limit. Because fruits are rinsed and brushed before pressing, residues found on fresh fruits may not be present at the same level after processing. It must be proven that pesticide residue levels in apple pomace are low before it is fed to ruminants. Fenarimol, 40 mg/kg Fenbutatin Oxide, 120 mg/kg Permethrin, and 80 mg/kg Propargite are the maximum residue levels in dried apple pomace, according to the (Codex Alimentarius, 2003).

When taken whole, apples, like other fruits, can be toxic to calves and sheep because they can block the oesophagus and cause interference. Because they like to pluck apples from the lowest branches, cattle grazing in apple orchards are in risk (Tran, personal communication).

2.9 Environmental Impact

Apple pomace is an agro-industrial by-product that is periodically dumped in fields or near processing facilities. It degrades quickly and has a high biological oxygen requirement (BOD). Because of its high water and fermentable sugar content, it spoils quickly and pollutes the environment; however, feeding apple pomace to cattle can help solve this problem (Crawshaw, 2004). Composting, production of fuel, and spreading apple pomace as mulch for landfilling are all options for disposing of the waste (Shalini *et al.*, 2010).

Pomace from apple can be used to make mulch or compost (Copas, 2004). To get a sufficient C:N ratio for composting, composting should be done in conjunction with a supply of C. Apple pomace has been demonstrated to be successful at suppressing weeds in the UK, and it might work as a germination medium for grass seeds in problematic situations like roadside ditches (Copas, 2004).

CHAPTER III

MATERIAL AND METHODS

3.1 Procurement and Sample Preparation

The determination of the nutritive value and the in-vitro digestibility of three varieties of apple pomace were determined in the animal nutrition laboratory of Niğde Ömer Halisdemir University's Ayhan Sahenk Faculty of Agricultural Science and Technologies in, Turkey. Fifteen (15) kg each of the apple cultıvars were procured from local market in Niğde, Turkey.

3.2 Preparation of Pomace

In this research, pomaces of three Apple varieties - Starking, Golden delicious and Granny smith in Niğde were used for in-vitro studies. The apple pomace samples were obtained by using electric juice extractor. The weighed samples were placed in the oven at a temperature of 50^{o}C for 48h. After 48h in the oven they were removed and placed on the bench for 24h to cool. The samples were weighed and grinded using a 1mm sieve at a speed of 1400rpm. They were then stored in pre-labelled plastic bags prior to chemical analysis.

3.3 Chemical Analysis of Samples

The dry matter (DM) and crude ash (XA) of apple pomaces were measured using the (AOAC, 1995) method. Nitrogen (N) content of Apple pomaces were determined using the Kjeldahl method (AOAC, 1995). Crude protein of Apple pomaces were estimated with N*6.25. The content of crude fiber (CF), neutral detergent fiber (NDF), and acid detergent fiber (ADF) in apple pomaces was determined using the Van Soest method (1991). All chemical analyses were carried out in duplicates.

3.3.1 Dry matter determination

Crucibles were cleaned and oven dried for 2h to a constant weight (W1). 1 g each of Apple pomace samples from the three Apple varieties were 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 105 ^{o}C for 24 h. After 24 h, they were taken out of the oven and placed in a desiccator to cool at room temperature for 20 minutes. The weight of the crucibles containing the samples were taken immediately and recorded as (W3). The dry matter was then calculated in percentage using Equation (3.1)

$$\% \text{ dry matter} = (W3 - W1)/W2 * 100 \quad (3.1)$$

3.3.2 Crude ash content

Crude Ash content was determined using the Muffle's furnace. The crucibles containing the Apple pomace samples used to determine dry matter with weight W3 were placed in the muffle's furnace for 4 h at a temperature of 600^{o}C. After ashing, the samples were taken out of the oven and placed in a desiccator to cool at room temperature for 20 minutes. The final weight of crucible and sample was then taken and recorded as W4. The ash content of each Apple pomaces was calculated using Equation (3.2). Organic matter was also calculated by subtracting the % ash content from 100%.

$$\% \text{ ash content} = (W4 - W1)/W2 * 100 \quad (3.2)$$

3.3.4 Crude protein

Crude protein of the samples was estimated using the Kjelroc digestion unit and Opsis liquid line Kjelroc analyzer made in Sweden. 1g each of pomaces from the three Apple varieties was put into an Opsis tube in duplicates. Each tube received two kjedahl tablets and 12 mL of H_2SO_4. The tubes were inserted in the digester block, and the protein digester was set for 60 minutes at 420 degrees Celsius. 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 (In 100 mL methanol, dissolve 100 mg bromocresol green and 70 mg methyl red separately) were attached to Kjelroc Analyzer. The machine was auto cleaned, and blank was determined before analyzing the samples for crude protein. The nitrogen factor in the Kjedahl program was set at 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.

3.3.5 Neutral detergent fiber (NDF)

The NDF was evaluated using the Ankom Technology ANKOM^{200} fiber analyzer, following the procedure outlined by (Goering and Van Soest, 1970). Seven F57 filter bags were weighed (W1) with an analytical scale after being labeled with a solvent resistant marker. The balance was reset to zero, and 0.55 g of Apple pomace sample was weighed into the filter bag straight. The bags were lightly tapped to disperse the samples evenly and prevent clumping. A heat sealer was used to close the bags within 4 mm of the top. One blank bag was also weighed to include in the run for determination of blank bag correction (C1). To generate one litre 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 were combined in 1 L distilled H_2O. The extraction method additionally included 20 g of sodium sulphite (Na_2SO_3) and alpha-amylase. The bags were placed 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 introduced into the fiber analyzer tank, with the suspender weight on top to keep it submerged. The ambient ND solution was produced and placed into the fiber analyzer tank in a volume of 2000 mL. To the solution in the vessel, 20g of Na_2SO_3 and 4.0 mL of alpha amylase were added. The agitation and heat buttons were turned on to begin the digesting process. Agitation was confirmed and the lid was closed. The timer had been set for 75 minutes. The heat and agitate buttons were turned off at the end of the extraction, and the drain valve was

opened to allow the hot solution to escape before the lid was opened. For the first and second rinses, 2000 mL of water at 90^{o}C with 4.0 mL of alpha-amylase was added to the vessel after the solution was released. Each rinsing lasted 5 minutes. 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 bags were gently tapped to remove surplus water once the samples were collected. The bags were soaked in acetone for 5 minutes before being totally air-dried on filter paper for 20 minutes. The bags were then dried entirely in the oven at 105°C for 2 hours before being placed in a foldable desiccant pouch for 20 minutes to cool at room temperature. With analytical balance aid, the bags were weighed (W3) and percentage NDF (as received basis) was calculated using Equation (3.3)

$$\% \text{ NDF (as received basis)} = (W3-(W1*C1))/W2*100 \quad (3.3)$$

Where W1= Weight of bag tare,

W2 = Weight of sample,

W3 = after the fiber extraction process, the dried weight of the bag with fiber,

C1 = Correction for blank bags (final oven – dried weight divided by the weight of the original blank bag)

3.3.6 Acid detergent fiber (NDF)

The ADF was evaluated using the Ankom Technology ANKOM^{200} fiber analyzer, following the procedure outlined by (Goering and Van Soest, 1970). Seven F57 filter bags were weighed (W1) with an analytical scale after being labelled with a solvent resistant marker. The balance was reset to zero, and the Apple pomace samples were weighed directly into the filter bag at 0.50 g (W2). The bags were lightly flicked to disseminate the samples evenly and prevent clumping. A heat sealer was used to close the bags within 4 mm of the top. In order to determine the blank bag adjustment, a blank bag was also weighed and included in the run (C1). In the bag suspender, the bags were placed on the trays, three bags on each tray. The trays were arranged in such a way that each one rotated at 120 °. The bag suspender was filled with all nine trays, and the bag suspender was then put into the fiber analyzer vessel. To keep the bag suspender submerged, a suspender weight was added on top. 20 g cetyl trimethylammonium

bromide (CTAB) was combined with 1 L of 1.00N H_2SO_4 to make an ADF solution. The fiber analyzer vessel was filled with 2 L of ADF solution and the lid was closed. The digestion process was set for 75 minutes and began when the agitation and heat buttons were pressed. 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 rinsing, the bags were removed and the surplus water was gently tapped out of them. The bags were then immersed in acetone for 5 minutes before being removed and completely air-dried on filter paper for 20 minutes. The bags were then dried entirely in the oven for 2 hours at 105 ℃. They were taken out of the oven after 2 hours and placed in a foldable desiccant pouch for 20 minutes to cool to room temperature. The bags were weighed W3 and percentage ADF (as received basis) was calculated using Equation (3.4)

$$\% \text{ ADF (as received)} = 100 * (W3 – (W1*C1))/ W2 \quad (3.4)$$

Where W1= Weight of bag tare,

W2 = Weight of sample,

W3 = after the fiber extraction process, the dried weight of the bag with fiber,

C1 = Correction for blank bags (final oven – dried weight divided by the weight of the original blank bag)

3.4 Total Phenolic Content

The total phenols (TP) content was measured using the Folin-Ciocalteau reagent and a photometric technique (Singleton *et al*., 1999). Gallic acid equivalents (mg GAE•100 g-1) were used to quantify the total phenol concentration in the apple pomace samples. A redox reaction is used to determine TPC levels. The determination of TPC is based on a redox reaction. Phenolic compounds in samples react with Folin-Ciocalteau reagent and turn it into an oxidized form. For the preparation of the standard graph, gallic acid is employed as a standard. Different concentrations of Gallic acid are used with ethanol (0.1-0.067-0.05-0.04 mg/mL) and their absorbance is read. The graph of absorbance is plotted against concentration. TPC are estimated following the graph (Singleton, 1999). For evaluation of TPC, extracts were obtained from fine powders of the three apple

pomace samples. Each sample was combined with 200 mL of 70% ethanol and 25 g of each were used. The samples were stored at room temperature for 24 hours. After that, the extracts were filtered using Whatman No 4 filter paper. In a rotary evaporator, supernatants were mixed with ethanol and evaporated under vacuum at 45°C. The remainder liquids were kept at -80°C until the Folin-Ciocalteu technique was used to determine total phenolic content. 5 mL of 0.2 N Folin-Ciocalteu reagent was combined with 900 mL of distilled water to make Folin Ciocalteu reagent. In 100 mL of the pomaces extract solution, 4 mL of saturated sodium carbonate (Na_2CO_3) solution (7.5 g/L) was added. After that, the samples were kept in the dark for two hours before their absorbance was measured in a spectrophotometer at 765 nm against a curve. Using an equation derived from a standard Gallic acid graph, total phenolic content was calculated as microgram Gallic acid equivalent per 100 g of dry weight.

3.5 In-vitro True Digestibility (IVTD)

ANKOM Technology's (Macedon, NY) DAISY II incubator was used to assess the IVTD of apple pomace samples from three cultivars of Apple. Daisy II Incubator has four incubation jars with a capacity of 2000 mL each. 1600 mL buffer solutions and 400 mL rumen liquor was added into each jar. The filter bags were placed in the jars.

3.5.1 Filter bags and samples are preparation

Ankom F57 filter bags were rinsed in acetone for 3 minutes before being air-dried fully. Following that, each bag was weighed, and the weight was recorded as (W1). The balance was reset to zero, and 0.5 g of sample (W2) was directly weighed into the filter bag. For the correction factor, empty filter bags were weighed and sealed as a blank. The bags were then heat sealed with an impulse bag sealer. The experiment was carried out in two batches, with triplicates of each of the three Apple pomace samples in each batch.

3.5.2 Making preparations for (combined buffer solution A and B)

The reagents and concentrations in Table 3.1 were used to make Buffer A and Buffer B solutions the night before the incubation. To 1330 mL of Solution A (Acidic solution),

266 mL of Solution B (Basic solution) was added. Using a pH meter, the exact amount of solution A to be added to solution B was adjusted to achieve a final pH of 6.8 at 39^{o}C.

3.5.3 Preparation of inoculum

The rumen liquid (inoculum) was taken from the rumens of two adult Holstein cattle that were slaughtered in a commercial abattoir in Niğde post-mortem. The collected rumen liquid was stored in two 2000 mL thermos flasks preheated at 39 ^{o}C and purged with CO_2. After adding two fistfuls of fibrous mat from the cattle's rumen to one of the flasks, the thermos flasks were transferred to the laboratory. The rumen liquid was placed into a hot blender preheated to 39 ° C.

Table 3.1. Composition of reagents for buffer solution A and B

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

For 30 seconds, the liquor was purged with CO2 gas, then mixed at high speed for 30 seconds. After that, the digesta was filtered through four layers of cheese cloth into a five-liter flask that had been warmed to 39 ° C. The remaining rumen fluid in the other thermos was similarly filtered into the same five-liter flask via four fresh layers of cheesecloth. Until the inoculum was introduced into the digestion jars, the rumen digesta in the five liter flask was continuously purged with CO2.

3.5.4 Incubation of samples

Each Daisy II digestion jar received 1600 mL of the resulting Buffer solution (A and B) and 400 mL of rumen inoculum, after which each digestion jar was purged with CO_2 for

30 seconds. The Filter bags with sealed blank bags for correction factor (C1) were then evenly distributed into three daisy II jars. The digestion jars were purged with CO_2 and tightly sealed. All of the digesting jars were placed in the Daisy II incubator, which had been pre-heated, and incubated for 48 hours at a temperature of $39^oC \pm 0.5$. The bags were taken from the digestion jars after 48 hours of incubation and rinsed with cold tap water with minimum mechanical agitation until the water was clear to stop the microbial activity. The bags were placed in sealable bags and stored in the refrigerator before NDF determination. The NDF of the samples was determined using an ANKOM^{200} fiber analyser (Ankom Technology) according to the procedure given by (Goering and Van Soest, 1970), and the weight after NDF determination was recorded as W3. The percentage in-vitro true digestibility on as received and dry matter basis was then calculated using Equations (3.5) and (3.6)

$$\% \text{ IVTD (as received)} = 100 - (W3-(W1*C1)/W2*100 \quad (3.5)$$

$$\% \text{ IVTD (DM)} = 100 - (W3-(W1*C1)/ (W2*DM) *100 \quad (3.6)$$

Where W1 = Weight of bag tare, W2 = Weight of sample, W3 = Bag weight (final) following sequential ND treatment and in-vitro analysis,

C1 = Correction for blank bag (final oven-dried weight/ original blank bag weight)

3.5.5 Neutral detergent fiber after in-vitro incubation

Neutral detergent fiber (NDF) of the digested samples was estimated using ANKOM^{200} fiber analyzer (Ankom Technology) following the method outlined by (Goering and Van Soest, 1970). The bag suspender was used to hold the bags carrying the digested samples. Each tray included three bags, and the trays were stacked in such a way that each tray was turned and properly put. The bag suspender was then introduced into the fiber analyzer vessel, followed by the suspender weight, which was placed on top to keep the bag suspender submerged. The ambient ND solution was produced and placed into the fiber analyzer tank in a volume of 2000 mL. To the solution in the vessel, 20 g of Na_2SO_3 and 4.0 mL of alpha-amylase were added. The agitation and heat buttons were turned on to begin the digesting process. The lid was closed after the agitation was

established. The timer had been set for 75 minutes. The heat and agitate buttons were turned off at the end of the extraction, and the drain valve was opened to allow the hot solution to escape before the lid was opened. For the first and second rinses, 2000 mL of water at 90 ^{o}C with 4.0 mL of alpha-amylase was added to the vessel after the solution was released. Each rinsing lasted 5 minutes. The third rinse, which lasted 5 minutes, was done with tap water without alpha-amylase. The samples were removed when the rinse process was completed, and the bags were lightly tapped to remove surplus water. The bags were soaked in acetone for 5 minutes before being totally air-dried on filter paper for 20 minutes. The bags were then dried entirely in the oven at 105°C for 2 hours before being placed in a foldable desiccant pouch for 20 minutes to cool to room temperature. With an analytical balance aid, the bags were weighed (W3) and percentage NDF (as received basis) was calculated using Equation (3.7)

$$\% \text{ NDF (as received)} = (W3-(W1*C1))/W2*100 \quad (3.7)$$

Where W1 = Weight of bag tare, W2 = Weight of sample, W3 = after the fiber extraction process, the dried weight of the bag with fiber,

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

3.5 Statistical Analysis

Validity of normality, homogeneity and independence for this research was ensured by subjecting the results of chemical parameters to Kruskal-Wallis' nonparametric one-way ANOVA while. All analyses were carried out on Jamovi, a statistical analysis package.

CHAPTER IV

RESULT

4.1 Nutritional Composition

The apple pomace samples obtained from the apple cultivars (Starking, Golden Delicious and Granny smith) were not significantly different in terms of the parameters measured (P>0.05) for chemical analysis (table 4.1). However, Granny smith pomace sample had the higher NDF value 29.8% than the other two apple pomace samples measured (figure 4.4). Golden delicious apple pomace sample had higher DM 95.3% and Ash 2.00% values compared to the other two apple pomaces measured (figure 4.1 and 4.2). Granny Smith apple pomace sample had higher CP 5.09% and ADF 25.3% content than the other apple pomace sample in comparison as shown in (figure 4.3 and 4.5) below.

Table 4.1. Chemical composition of apple pomace samples

S/n	Sample	ADM	DM	XA	CP	NDF	ADF
1	Golden Delicious	25.19±0.019	95.30±0.031*	2.00±0.172*	2.57±0.074	28.00±0.309	22.70±1.020
2	Starking	27.24±0.133*	95.10±0.028	1.47±0.082	2.02±0.021	20.70±0.291	15.90±0.131
3	Granny Smith	25.69±0.066	94.10±0.031	1.90±0.061	5.09±0.153*	29.80±0.360*	25.30±0.153*

At P > 0.05 all three apple pomace samples were not statistically different from each other.
***** shows the sample that ranked highest for each parameter
DM = Dry matter, XA = Ash content, CP = Crude protein, NDF = Neutral detergent fiber ADF = Acid detergent fiber, ADM = Air dry matter

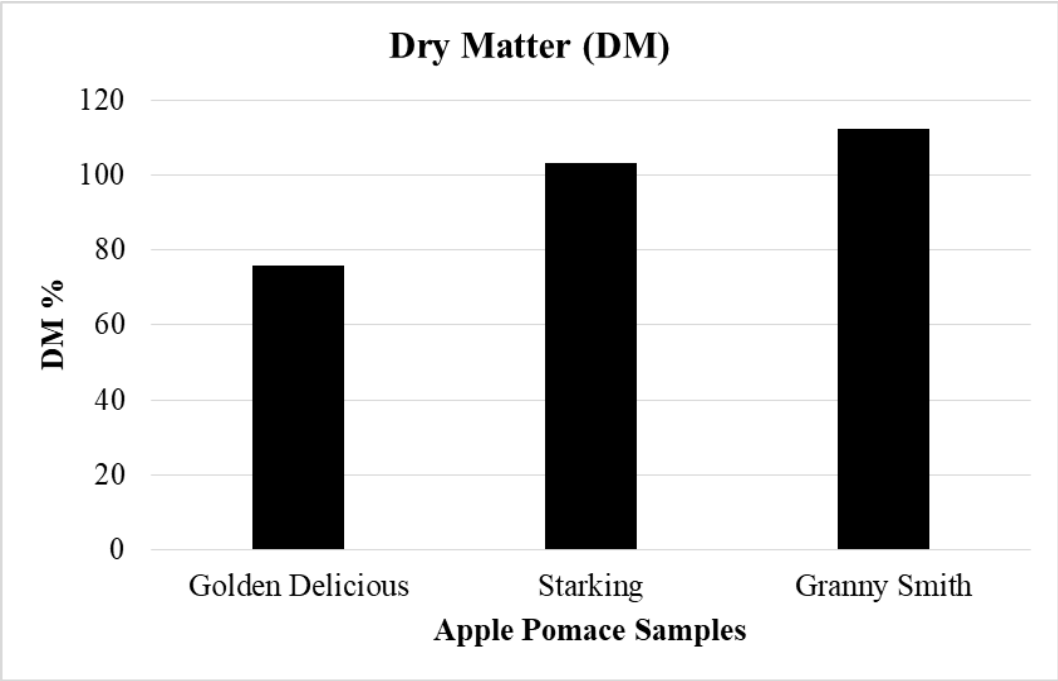

Figure 4.1. Dry matter of apple pomace samples

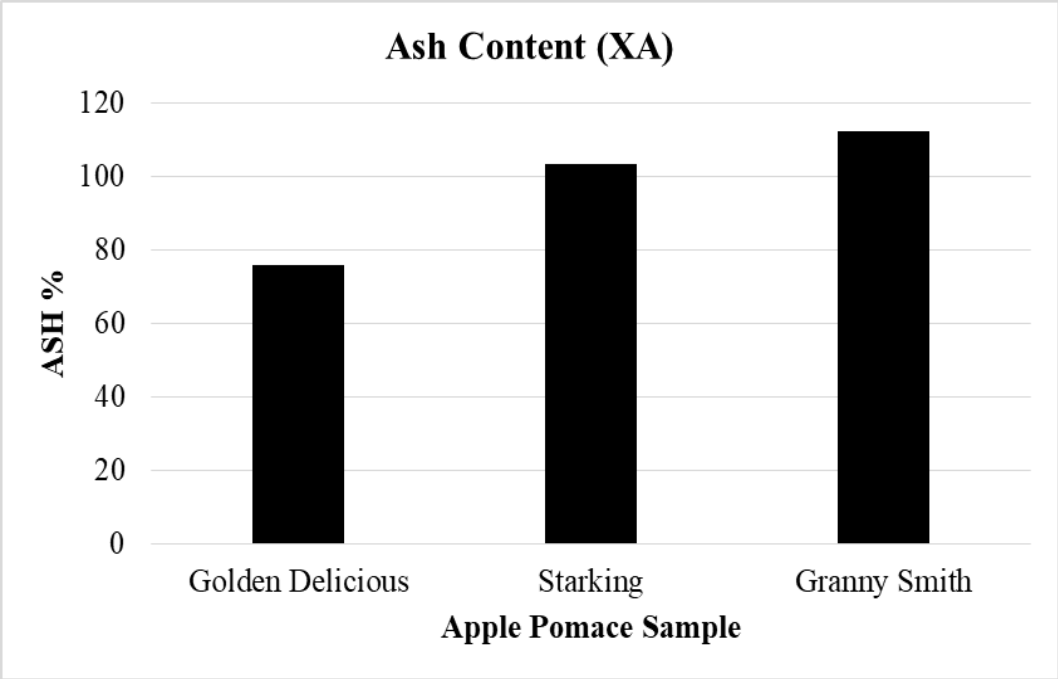

Figure 4.2. Ash content of apple pomace samples

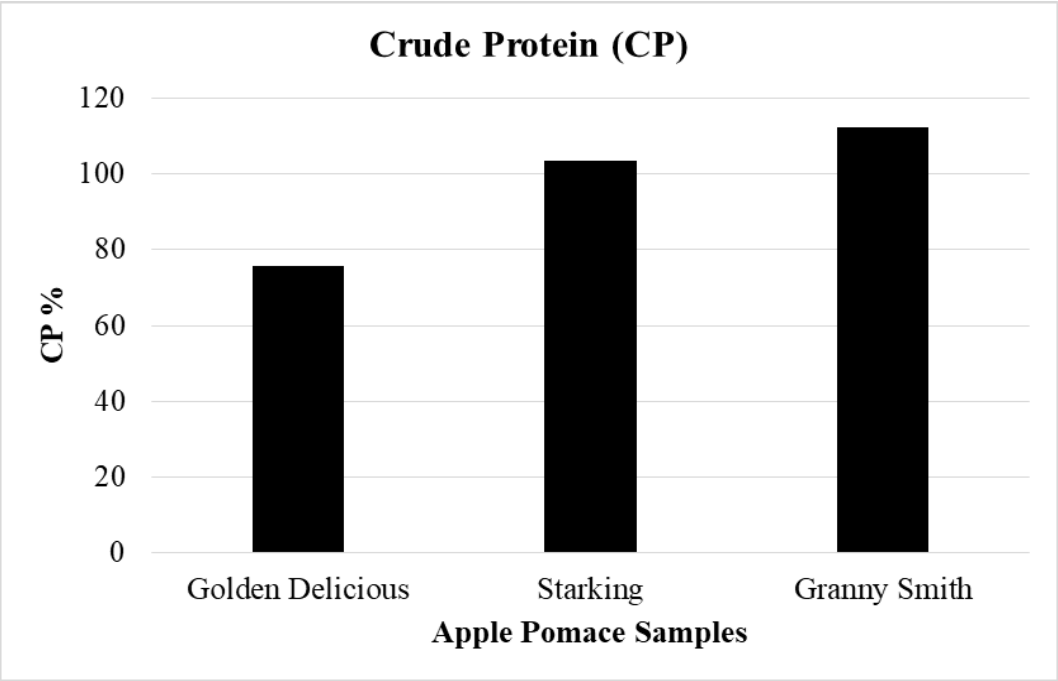

Figure 4.3. Crude protein content of apple pomace samples

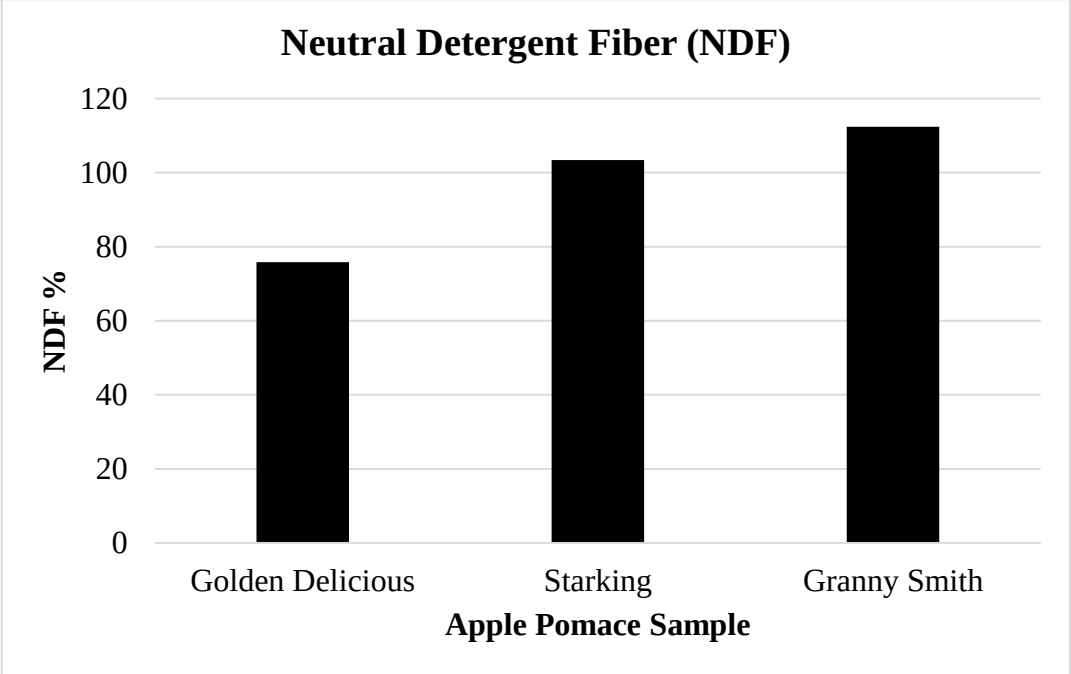

Figure 4.4. Neutral detergent fiber of apple pomace samples

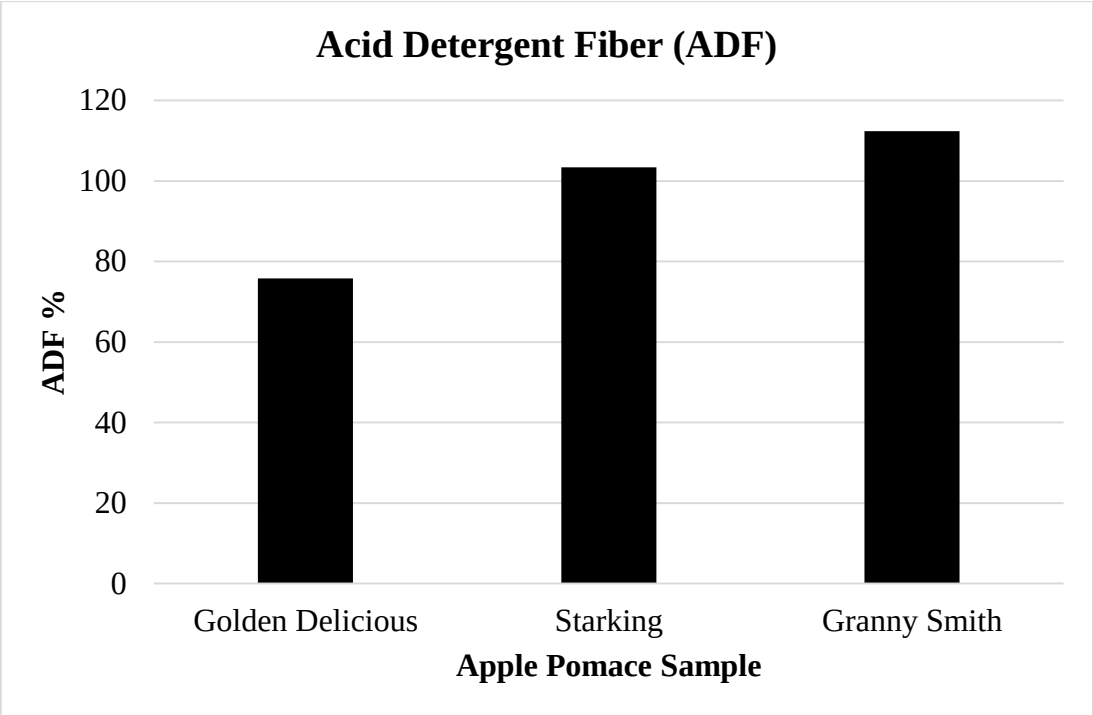

Figure 4.5. Acid detergent fiber of apple pomace samples

4.2 Total Phenolic Contents

The apple pomace samples were not significantly different from each other (P>0.05) for total phenolics content. However, Granny smith have the highest total phenolic content compaired to Starking and Golden delicious as shown in table 4.2 below.

Table 4.2. Total phenolic contents of apple pomaces

Unit	Sample	Total Phenolic Contents (mg GAE/100g)
1	Golden Delicious	75.8
2	Starking	103.4
3	Granny Smith	112.4

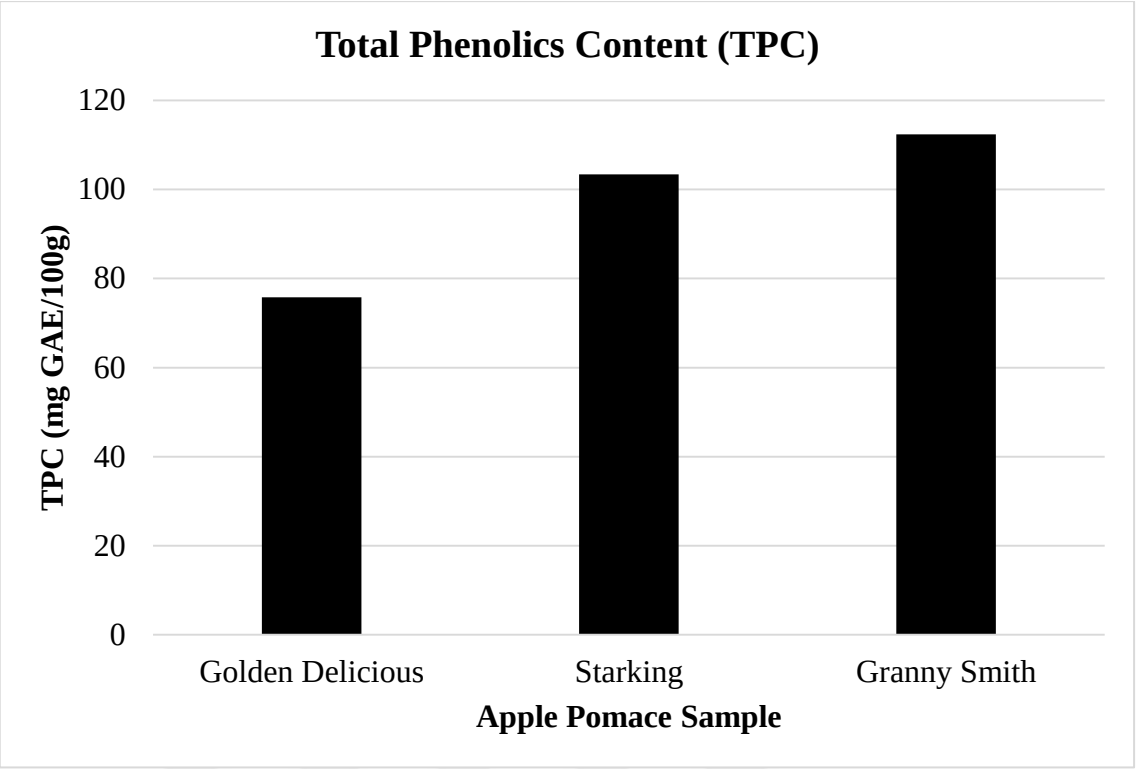

Figure 4.6. Chart showing the total phenolics content (TPC) of apple pomace samples

4.3 In-vitro Digestibility Trial

Apple pomace samples were significantly different at $p < 0.05$ for in-vitro true digestibility. Starking apple pomace sample had higher IVTD (as fed) 92.36% (figure 4.7). IVTD (DM) 92.23% was also higher in Starking apple pomace (figure 4.8) and NDFD (%) 63.12% value (figure 4.9).Granny Smith sample had significantly different ($p < 0.05$) NDFD from Golden Delicious and Starking samples (table 4.3).

Table 4.3. In-vitro digestibility of apple pomaces samples

Unit	Sample	IVTD (As fed)	IVTD (DM)	NDFD
1	Golden Delicious	88.17 ± 0.789^{c}	87.47 ± 0.782^{b}	57.11±4.13
2	Starking	92.36 ± 0.386^{a}	92.23 ± 0.311^{a}	63.12±1.81
3	Granny Smith	80.59 ± 0.738^{b}	79.04 ± 0.752^{c}	31.19 ± 3.01^{a}

IVTD (DM) = In-vitro true digestibility dry matter; NDFD = Neutral detergent fiber digestibility
Note: a-c superscripts represent points of statistical significance at P<0.05

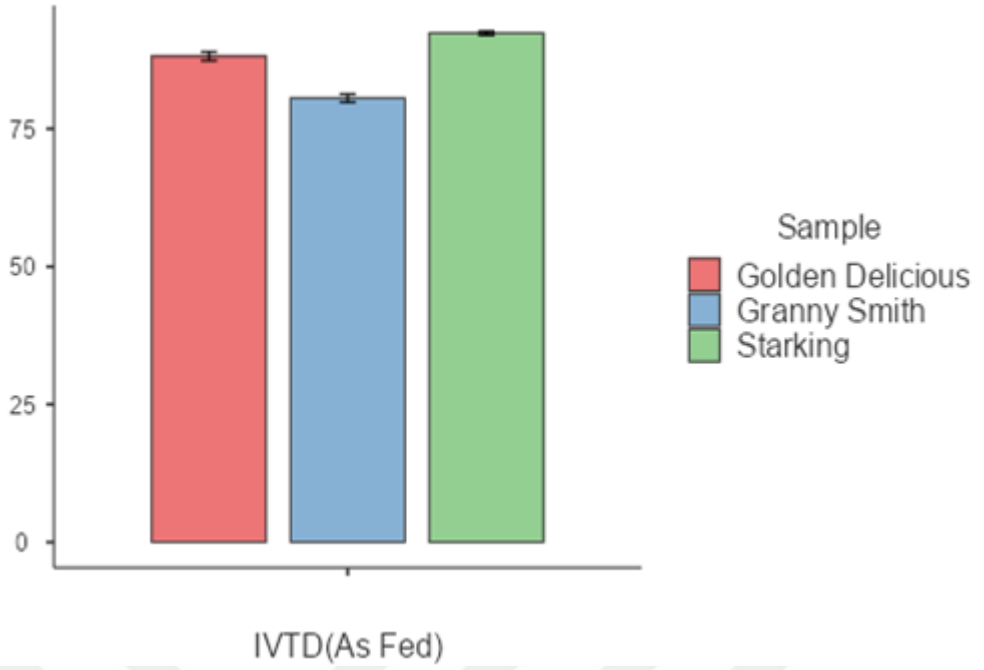

Figure 4.7. Chart showing in - vitro true digestibility IVTD (as fed) of apple pomace samples

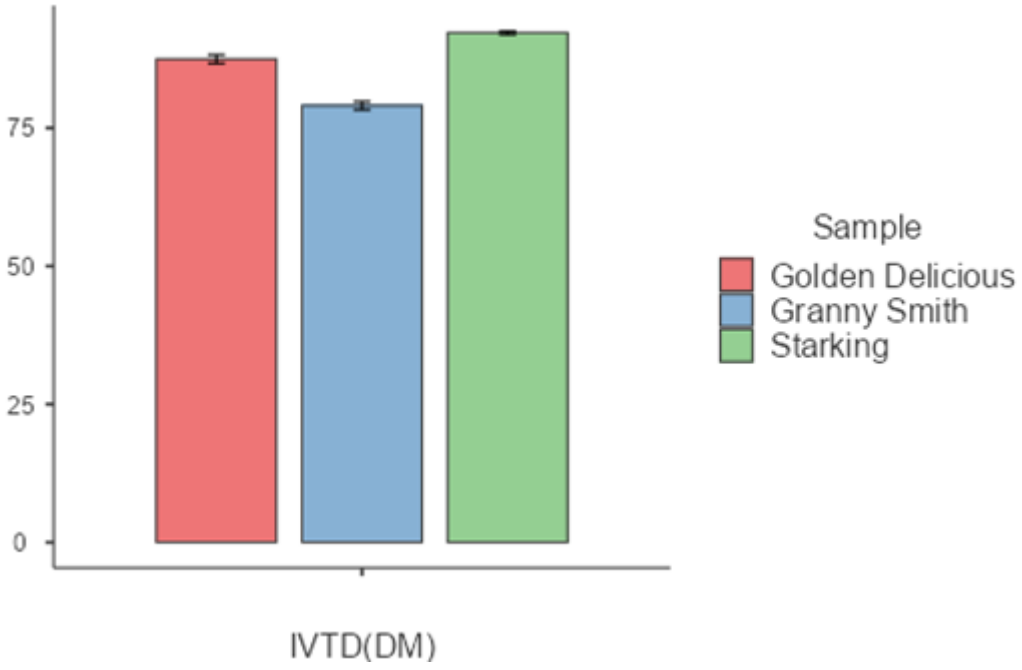

Figure 4.8. Chart showing in - vitro true digestibility IVTD (DM) of apple pomace samples

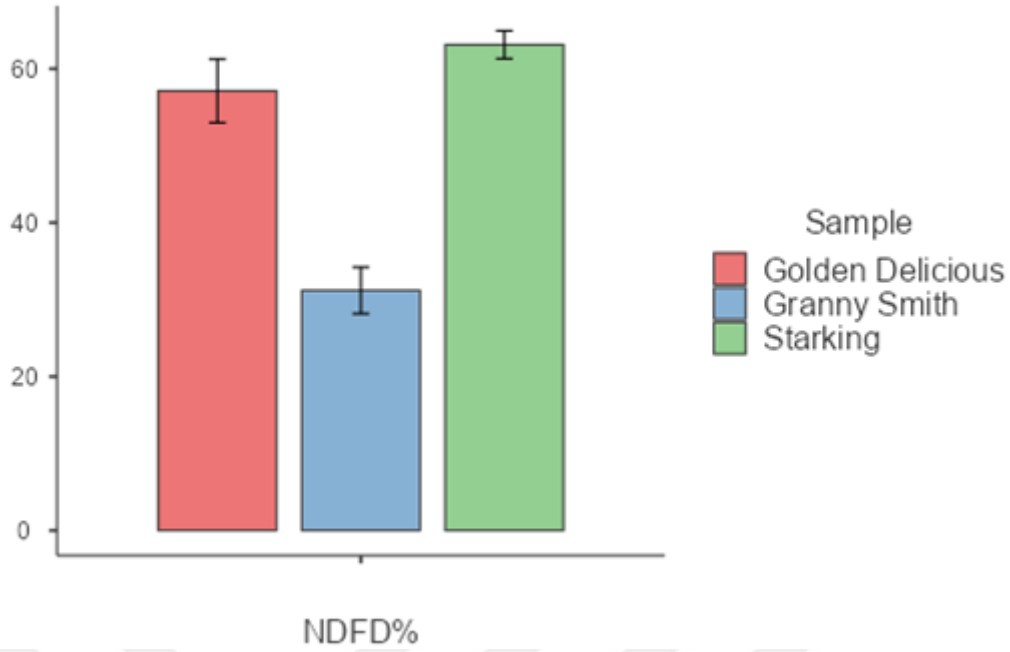

Figure 4.9. Chart showing neutral detergent fiber digestibility (NDFD) of apple pomace samples

CHAPTER V

DISCUSSION

5.1 Nutritional Composition

The apple pomace samples analysed in this study were not significantly different in terms of the parameters measured (P>0.05) for chemical analysis. However, the DM 94-95% obtained from the three apple pomace samples was higher than 91.2% reported by (Heuzé *et al.*, 2020). The 2-5% CP obtained in this study was lower than 8% CP reported by (Heuzé *et al.*, 2020). However, (Albuquerque, 2003) reported 3.7% and 1.5% apple pomace CP value was reported by (Jin *et al.*, 2002) which falls in the range of 2-5% CP value obtained in this study. The Ash content of the three apple pomace samples 1-2% analysed in this study is similar to 2.1% reported by (Heuzé *et al.*, 2020). The NDF 20-29.8% values obtained in this study was the same with 30-68% reported by (Afzal *et al.*, 2015). (Preston, 2014) reported 36% NDF value which is higher but in close range with the values obtained in this study for the three samples of apple pomace from three different apple varieties analysed. However, (Heuzé *et al.*, 2020) reported 65.1% which is higher than the values obtained in this study. The 15-25.3% ADF values of apple pomace samples obtained in this study fall within the range of 25.00–43.20% reported by (Afzal *et al.*, 2015) but is lower than 57.7% reported by (Heuzé *et al.*, 2020), but in close range 27% reported by (Preston, 2014). Therefore, it can be stated that the nutritive values of apple pomace samples from three different apple varieties analysed (Starking, Golden delicious and Granny Smith), was within the range established by other authors. Slight discrepancies could be attributable to the fact that apple pomace has different proportions of skins, pulp, core, seeds, and juice, and apple composition changes based on varietals, ripeness, harvest season, and other factors (Grigoraş, 2012).

5.2 Total Phenolics Content (TPC)

The TPC analysed in this study showed that Granny Smith had higher TPC than Starking and Golden delicious (figure 4.6). In this study apple pomace samples from Golden delicious, Starking and Granny smith had TPC of 75.8, 103.4 and 112.4 mg

GAE/100g respectively which was lower than the value of 144, 143 and 132 mg GAE/100g reported by (Er and Özcan, 2010) and 86.3, 131.1 and 121.0 mg GAE/100g as reported by (Vrhovsek *et al.*, 2004). However, (Bai *et al.*, 2010) reported a value of 62.7 mg GAE/100g and as well (Adil *et al.*, 2007) reported 47 mg GAE/100g which were lower than all the values found in this study. According to (Persic *et al.*, 2017), the total polyphenol concentration of apple pomace is 19–50 mg gallic acid/100 g d.m. In this context, the overall polyphenol concentration in the studied apple pomaces was within the range described by previous authors, and the minor variations could be attributed to their origin. According to (Cetkovic *et al.*, 2007), the wide range of polyphenols identified was mostly due to diverse apple types and extraction circumstances (type of medium, temperature, pH, time). Environmental factors may have a significant impact on the amount of polyphenols present (Jakobek *et al.*, 2020). Different farming methods, such as conventional, integrated, or organic farming, may have an impact on the polyphenol profile (Santarelli *et al.*, 2020). The geographical location of orchards can influence the color of apples and thus the quantity of pigments (Yuri *et al.*, 2019).

5.2.1 In-vitro true dry matter digestibility

There appeared to be limited studies and literature on the in – vitro true dry matter digestibility of apple pomace samples from different apple cultivars. However, the IVTD (DM) of 80.59 – 92.36% obtained in this study is similar to 84 – 90% reported by (Kafilzadeh *et al.*, 2008; Anrique *et al.*, 2002) and 82 – 84% reported by (Singh *et al.*, 1992). A study conducted by (Tagliapietra *et al.*, 2015) for the digestibility of using the gas production method, fresh apple pomace yielded 98% after 48h incubation which is close to the range of values obtained in this studies. When evaluated on sheep, *in-vivo* organic matter digestibility of nitrogen-treated apple pomace silage varied from 70 to 78 % (Alibes *et al.*, 1984), which is consistent with current *in-vitro* values (Alibes *et al.*, 1984). *In vivo* dry matter and organic matter digestibility of dried apple pomace were 66.8% and 69.9%, respectively, when evaluated in sheep with just urea as a supplement (Taasoli *et al.*, 2008) were lower than the range of values obtained in the current study. Apple pomace might completely replace barley in an alfalfa hay-based diet, according to in-vitro digestibility estimations (Khatooni *et al.*, 2014). Because of its high soluble component, apple pomace has a high DM digestibility.

5.2.2 Neutral detergent fiber digestibility

In this study, Starking apple pomace sample had higher 63.12% NDFD than Granny Smith 31.19% and Golden delicious 57.11% (Figure 4.10). Limited studies have been carried out on the NDFD% of apple pomace samples. However, an in-vivo studies involving apple pomace reported 68.4% NDFD which is within the range of values 31.19 – 63.12% obtained in this studies (Ahn *et al*., 2002).

CHAPTER VI

CONCLUSION

For the chemical analysis, there were no statistically significant differences between the apple pomace samples. However, Granny smith pomace sample have the highest NDF value than the other two apple pomace samples measured while Golden delicious apple pomace sample have highest DM and Ash compared to the other two apple pomaces measured, and Granny Smith apple pomace sample have highest CP and ADF content than the other apple pomace samples. There were no statistically significant variations in in-vitro digestibility characteristics tested between the apple pomace samples except for the NDFD in which at $p < 0.05$ the Granny Smith sample had significantly different NDFD from Golden Delicious and Starking samples (table 4.3). However, among the parameters measured, IVTD (DM), IVTD (as fed), and NDFD (%) values was found to be highest in Starking apple pomace sample. Among the cultivars studied, Granny Smith has the highest overall phenolic content.

The use of readily available and cheap sources of agro-industrial feeds such as apple pomaces is an important aspect to consider in ruminant nutrition. This research is important in providing additional knowledge on the nutritive value and in – vitro digestibility of apple pomaces of different apple varieties as well as a provision of opportunities to researchers and nutritionist on the potentials of these agro-industrial bye – products to replace or at least modify conventional feed ration of ruminants. One of the most profitable solutions for apple processing companies to overcome the present feed scarcity is to use apple pomace as livestock feed.

This implies that, the abundant number of apple cultivars as well as the diverse uses will continue to provide a good source of agro-industrial wastes. These would enable researchers to explore the opportunities for improved ruminant ration, resource-efficient and eco – friendliness. It is recommended that further studies should be carried out to define the use of apple pomace in ruminant ration.

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CURRICULUME VITAE

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