T.R.
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL SCIENCES AND TECHNOLOGIES

DETERMINATION OF RUMEN FERMENTATION KINETICS AND *IN-VITRO* DIGESTIBILITY OF SUGAR BEET PULP PRODUCTS ADDED IN CATTLE DIETS

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Supervisor
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M. Sc. Thesis

August 2023
KAYSERİ

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SCIENTIFIC ETHICS SUITABILITY

İm Idil Elmi DAHIR, hereby declare that this piece of work is the result of my research and that this work has never been submitted anywhere for any degree. All the sources of information used were obtained by academic ethics and have been duly acknowledged.

Idil Elmi DAHIR

This master's thesis was written upon the topic "**Determination of Rumen Fermentation Kinetics and *In-Vitro* Digestibility of Sugar Beet Pulp Products Added in Cattle Diets"** was prepared with accordance Erciyes University's graduate Thesis Writing Directive

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Idil Elmi DAHIR

Kayseri, September 2023

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Erciyes University, Graduate School of Natural and Applied Science
M.Sc. Thesis, August 2023
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ABSTRACT

In this thesis, two studies were conducted to determine the effects of sugar beet pulp (SBP) products and the addition of pectin to dairy cow feeds. In the first study, the chemical compositions, *in vitro* gas production (GP), methane production (GP) and dry matter (DM) and organic matter digestibility (OMD) and energy values of pectin, cellulose, carboxymethyl cellulose (CMC) and dietary fiber obtained from SBP. In the second study, chemical composition, total GP and MP, *in vitro* dry matter digestibility (DMD) and OMD values and energy values of addition 0, 0.5 and 1% pectin to dairy cattle rations were determined. In the first study, there were no significant difference between SBP and its products in terms of DM and OM ratios, but there were differences in crude protein, fat, ash and ADF and NDF of products ($P<0.05$). The 6, 12 and 24 hour GP of the samples were significantly different and the highest and lowest GP were obtained in the pectin and CMC products, respectively ($P<0.01$). There was no significant differences in MP production. The DMD and OMD of pectin were significantly higher than the other products ($P<0.01$). Metabolic energy (ME, MJ/kg dry matter) and net energy lactation (NEL) values of sugar beet pulp, dietary cellulose and pectin were significantly higher than those of cellulose and CMC products ($P<0.01$). In the second study, there were significant differences in DM, crude protein, fat, ash, NDF, ADF and organic matter content of feeds. There were significant differences between the products that added 0, 0.5% and 1 pectin to the dairy cattle ration ($p<0.01$). Gas production of pectin was significantly higher than the other products ($P<0.05$), and there was no significant difference between the products in terms of MP. Pectin's DMS and OMS ratios, and ME and NEL values were significantly higher than the other groups ($P<0.01$).

Keywords: sugar beet pulp, by-products, increasing value, global warming, sustainability

SIĞIR YEMLERINE KATILAN ŞEKER PANCARI KÜSPESI ÜRÜNLERININ RUMEN FERMENTASYON KINETIĞI VE IN-VITRO SINDIRILEBILIRLIKLERININ BELIRLENMESI

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ÖZET

Bu tez çalışmasında, şeker pancarı posası (SPP) ve ürünlerinin ile süt ineği yemlerine pektin ilavesinin etkilerini belirlemek amacıyla iki çalışma yapılmıştır. İlk çalışmada pektin, selüloz, karboksimetil selüloz (CMC) ve diyet selülozun kimyasal bileşenleri, *in vitro* gaz üretimi (GÜ), metan üretimi (GÜ) ve kuru madde (KM) ve organik madde sindirilebilirliği (OMS) ile enerji değerleri incelenmiştir. İkinci çalışmada, süt sığırı rasyonlarına %0, 0,5 ve 1 pektin ilavesinin kimyasal bileşimi, toplam GP ve MP, in vitro kuru madde sindirilebilirliği (KMS) ve OMS değerleri ile enerji değerleri belirlenmiştir. İlk çalışmada SBP ve ürünleri arasında KM ve OM oranları açısından anlamlı bir fark bulunmazken, ürünlerin ham protein, yağ, kül ve ADF ve NDF'leri arasında farklılıklar önemli bulunmuştur ($P<0.05$). Örneklerin 6, 12 ve 24 saatlik GÜ'leri önemli ölçüde farklılık göstermiş ve en yüksek ve en düşük GÜ sırasıyla pektin ve CMC ürünlerinde elde edilmiştir ($P<0.01$). MÜ üretiminde önemli bir fark bulunmamıştır. Pektinin KMS ve OMS'si diğer ürünlere göre anlamlı derecede yüksekti ($P<0.01$). Şeker pancarı küspesi, diyet selüloz ve pektinin metabolik enerji (ME, MJ/kg kuru madde) ve net enerji laktasyon (NEL) değerleri, selüloz ve CMC ürünlerinden önemli ölçüde yüksek bulunmuştur ($P<0.01$). İkinci çalışmada, yemlere %0, 0,5 ve 1 pektin ilavesinin KM, ham protein, yağ, kül, NDF, ADF ve organik madde içeriklerinde önemli farklılıklar bulunmuştur. Süt sığırı rasyonuna %0, %0,5 ve 1 pektin ilave edilen ürünler arasında önemli farklılıklar bulunmuştur ($p<0,01$). Pektinin gaz üretimi diğer ürünlere göre anlamlı düzeyde yüksek ($P<0.05$) ve MP açısından ürünler arasında anlamlı bir fark bulunmamıştır. Pektin'in KMS ve OMS oranları ile ME ve NEL değerleri diğer gruplara göre anlamlı derecede yüksek bulunmuştur ($P<0.01$).

Anahtar kelimeler: şeker pancar posası, yan ürünler, değerini artırma, global ısınma, sürdürülebilirlik

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INTRODUCTION

The sugar-producing industry generates significant quantities of sugar beet pulp (SBP) as a by-product during the processing of sugar beet. This waste material has considerable value due to its arrangement, which includes cellulose, hemicellulose, and pectin. The process of converting SBP into value-added compounds is achieved by many methods, including hydrothermal procedures, acid hydrolysis, and enzymatic hydrolysis. The process of enzymatic hydrolysis is a cost-effective and environmentally favorable technique that enables the biological transformation of beet pulp into easily fermentable carbohydrates, thereby facilitating the production of value-added products. Although beet pulp has traditionally been utilized as livestock feed for ruminant species, advancements in biotechnological processes have revealed its capacity as a valuable Raw material for manufacturing of various biofuels, biodegradable plastics, bio hydrogen, lactic acid, alcohols, citric acid, cell-specific proteins, microbial enzymes and Oligosaccharides of the pectin molecule (Usmani et al., 2022).

When SBP is stored under unsuitable conditions, it is exposed to unwanted aerobic and anaerobic fermentation and has negative effects on the environment (Tatli Seven et al., 2021). For this aim, it is essential to properly ensilage these products. At the same time, work continues on the production of new products from SBP, such as many agro-industrial products, and their conversion into more valuable products (Ülger et al., 2015). On the other hand, as an industrial byproducts SBP can be economically viable in dairy and feedlot farms. However, high inclusion rates in the daily diets may reduce feed intake, performance (Münnich et al., 2017), diarrhea and intestinal problems (Özen et al., 1999).

The SBP is mostly composed of cellulose, hemicellulose, and pectin and is a valuable commercial by product. SBP serves as a building block in the bioproduction of enzymes produced by microbes: single cell protein, ethanol, pectin oligosaccharides, a form of hydrogen, lactate, ferulic acid, and methane or biogas. Throughout the fermentation, SBP can also play the role of a carrier for the immobilization of cells (Puligundla and Mok, 2021).

Global warming has been one of the most pressing issues in recent years. Additionally agricultural industry also contributes to global warming about 16%. As agricultural activities the contribution of the livestock sector to global warming is estimated around 9%. Methane gases produced by animals provide the greatest contribution to the greenhouse gas formation originating from the industry. For this purpose, a significant amount of work is being done on the reduction of methane gas produced by feeding ruminant animals (Koneswaran and Nierenberg, 2008). These studies are in the form of evaluation of feed additives and feed combinations to reduce gas production in the rumen. To decrease gas production from livestock production and decrease detrimental effect on environment lots of efforts under apply. For this reason, many feed additives, especially biotechnological products, have started to be produced for feeds in the last years (Dey et al., 2021; Bačėninaitė et al., 2022). In addition, important studies are carried out on finding suitable combinations of currently used feeds and adding phytogenic compounds in plants to feeds (Chaturvedi et al., 2015; Cobellis et al., 2016). In addition, efforts are underway to find solutions that reduce methane production from some feeds and at the same time maintain yield without changing it. Sugar beet factories are an important economic industrial area in Turkiye, and they also produce a significant amount of waste by-products to the environment. One of these by-products is SBP and it is widely used in animal feeding in provinces where sugar factories are close. In order to increase the value of sugar beet pulp, it is necessary to separate some chemical components and determine the potential of these products to be used as additives in animal nutrition.

Therefore, in this study, it is determined that the nutritional values of some products which are produced from SBP and gas production potentials in dairy cows.

CHAPTER 1

GENERAL INFORMATION AND LITERATURE REVIEW

In 2022, sugar production in the world total 170 512 000 tons, and share of EU 10,02% and Turkiye 6.82% in total sugar production of world (Turkseker 2021 Sektor report). Sugar beet production in the USA 32 574 000 thousand ton in 2022 (www.nas.usda.gov.tr) and in the European Union countries produces 50% of sugar in the world (https://agriculture.ec.europa.eu/) and in the Turkiye 17767086 thousand tons. Wet sugar beet pup production is about 30% of total processed sugar beet. Therefore its production is about 6 million thousand tons per year in Turkiye. (Simić et al., 2021) determined that quantity of fiber in SBP varies from 73% to 80%. SBP is a product of the sugar industry.

Michel et al. (1988) dried sugar beet pulp contain 7-8% protein, 21.5-23% uronic acids, 1-2% lignin, 7.5-12% ash, 22-24% cellulose and 0.5% residual sucrose, 26-32% hemicelluloses. Also, the researchers found that the wet beet pulp did not lose its nutritional value after drying and had a similar nutrient content to the initial samples.

Habeeb (2017) noted that SBP have highly digested fiber that is suitable for ruminants because it promotes acetate generation and preserves rumen health. SBP is consumed by livestock as fresh, dried and silage and highly palatable feed resource. SBP is not high in sugar beet pulp and other non-structural carbohydrate; also it is rich fiber and includes 0.5% phosphorus and 0.8% calcium. The fibrous residues of SBP mainly consist of highly digestible cellulose. SBP is high volume in per liters and so it is cubed and pelleted and transported for livestock farms. SBP has been using in beef cattle diets for roughage replacement and physical fullness of the rumen.

Kelly, (1983) reported that dried molassed SBP is including 12. metabolisable energy (ME) kcal/kg and protein 79 g per kg of dried SBP. Although crude fiber contain is high however it has high digestibility and less depressive for rumen function and no acidosis problem in ruminants. SBP may help to increase milk fat due to its highly digestible cellulose. It can be mixed with other feed and feed additive materials such as urea and be increased its feed value.

When giving fresh SBP to animals, it can be mixed with some other feeds because it causes some digestive problems, especially diarrhea. In a study (ülger et al., 2018), SBP mixed with citrus and peach pomaces and compared their nutrient composition and silage quality. Even though the amounts of ADF and crude cellulose contents were different however, neutral detergent fiber (NDF) didn’t change. Researchers found that SBP silage has higher DM digestibility (74.41%) and organic matter, as well as a high level of digestible energy (DE) (2.67 Mcal/kg DM) and net energy lactation (NEL) (1.61 Mcal/kg DM).

Usmani et al. (2022) reported that SBP which may be used to produce hemicellulose, cellulose and pectin. Acidic, hydrothermal, and enzymatic hydrolysis may convert SBP into value-added products. Biochemically converting SBP into fermentable sugars is cheap and environmentally benign. Modern biotechnological advances have made it a feedstock for biofuels, biodegradable polymers, bio hydrogen, and platform chemicals. https://books.rsc.org/books/edited-volume/1816/chapter-abstract/2053225/An-Investigation-on-the-Functional-Properties-of?redirectedFrom=fulltext

In dairy cow diets, when SBP used it may cause an economic dairy production, however its high ratio in diets result in lower performance and feed intake. SBP may enhance milk fat but not milk yield. Feeding with BPS interacted for molar percentages of propionate, acetate, butyrate and propionate. Also BPS may enhance net food production variables (Münnich et al., 2017). In difference to this author, Hemingway et al. (1986) reported that feeding with 5 kg dry matter of unmolassed pressed SBP caused a reduction in milk fatty substances with a rise in milk production; however, (201 g DM kg^{-1}) did not affect milk output and protein.

(Münnich et al., 2017) did and meta-analyses from 34 published papers between 1990 to 2016 years with 103 dietary in dairy cows. The researchers found that animal productivity high and SBP high in diets they caused some negative effects on rumen organic acids (acetic, butyric and propionic acids). In conclusion, it may be beneficial in cows if it does not surpass 3.5% the additional SBP in diet low ratio and feed intake level of the body weight.

Nemati et al. (2020) reported that feeding of high-producing dairy cows with SBP replace (5 to 15 % of DM) of corn and barley did not change DM and crude protein consumption, nutrient digestibility, sorting and chewing activities. During the comparation of low SBP and high SBP the starch intake of the cow decreased into 5.70 and 7.43 respectively. The addition of 5% and 15% SBP increased ruminal pH 4h after feeding (6.20 vs. 6.39) and milk fat content (2.92 vs. 3.15%). SBP substitution in diet barley or corn grains in high producing cows caused a decrease in starch intake, increase in ruminal pH after 4 h of feeding.

In another study (Mahmoud et al., 2019), replacing SBP with yellow corn in diets didn't change DM, OM CP and ash of diets. However, crude cellulose content of diets increased with increase of SBP in diets, NDF was higher and non-fibrious carbohydrates was lower in dried SBP added diets. Fully replacing of corn with SBP increased the number of the protozoas and bacteria inside the rumen. Throughout the experiment acetate: propionate ratio increased linearly with dried SBP addition. Researchers claimed that dried SBP can be replaced partially or fully without any negative effect dry matter digestibility and on rumen parameters. In goat trials, it is showed that SBP replacing with corn did not effect on milk production and milk fat but reduced body fat accumulation (Ibáñez et al., 2015).

García-Velásquez and van der Meer (2023) reported that SBP when use for animal feed or fertilizer its price is lower than biorafinery feed supplements (pectin derivatives oligosaccharides) and commodity chemicals (e.g. C_6H_4 $(CO_2H)_2$) values. The market price of the oligosaccharides derived from pectin and by product value determines economic viability.

Adiletta et al. (2020) and (Usmani et al., 2022) reported that SBP-valorization starting sugar industry may turn some valuable product and get much more income than conventional use for animal feed. When it separated some valuable compounds should be considered to produce pectin, cellulose and hemicellulose, Biofuels, bio-hydrogen, reusable materials for plastics, organometallic compounds, alcohols, single-cell proteins, and microbial enzymes are all products derived from microorganisms.

Evans et al. (2016) SBP in addition to $NaHCO_3$ can be used an amount of the cereal in dairy cows' nutrition. When fresh SBP replaced the grains in the diets 0, 8, 16, or 24% in DM basis, did not change in milk production, milk’s fat and protein ratio among the diets. The effectiveness amounts feed conversion efficiency; for each treatment, the dry matter intake and dry matter intake for energy-corrected milk were comparable. These results suggest that SBP with $NaHCO_3$ may be used for the replacement of up to 24% of the total minimum requirement in dry matter in the diets of dairy cows without negative effects.

Kajikawa et al. (1990) evaluated to assess the ruminant features and lactating cow production of two separate groups of Holstein cows with varied ruminal acetate to propionate ratios. After receiving the control diet, the low acetate/propionate ratio group had a higher volatile fatty acid concentration and a low ruminal pH than the high acetate/propionate ratio group, despite similar fiber and carbohydrate intakes and the ability to buffer. Even though sugar beet pulp supplemented some of the main ingredients in the diet of the control group, the ruminal and lactating performance of the HA group was not affected.

Gurbuz and Kaplan (2008) compared the chemical composition, metabolic energy, and OMD of mixtures of sugar beet leaves and maize silage to corn silage and sugar beet leaf silage, respectively. The processing of sugar beet leaves together with whole-crop corn substantially altered the combinations' chemical composition, pH, OMD, and determined ME value in comparison to pure silage. The total gas production in sugar beet leaf silages was lower than corn silages (50 vs 247.5). Making silage with sugar beet leaves and corn ay improve silage quality and the optimal proportion for mixed silages was 50:50 %.

Evans and Messerschmidt (2017) dairy cows usually get grains and highly digested byproducts in addition to grass to help them produce milk. Also Sugar beets are fairly strong, and their returns on dry matter returns are higher than those of most food crops. But sugar has been left out of many feeding programmers because it is thought that it lowers digestive system pH, fiber breakdown, and microbial output.

Hellwing et al. (2017) in his study, SBP was ensiled with or without addition of silage additives. At the same time, the sugar beet silage produced was replaced with corn silage and fed to dairy cows. Researchers reported that when sugar beet was fed with added silage additive, milk yield decreased slightly, but the differences between sugar beet silages without silage additives and there was no significance between groups fed maize silage.

Boguhn et al. (2010) noted that diets (TMR) contained or not contained SBP silage, maize silage, 118 days of feeding. The DM intake of cows was substantially decreased by SBP silage, but a digestibility study revealed a greater organic matter digestibility and metabolizable energy level in the TMR with SBP silage. The kinetics of gas production *in vitro* revealed that a lower fermentation intensity in the TMR with SBP. The TMRs did not differ in their in vitro production of short-chain fatty acids. However, the addition of SBP silage to the diet decreased the efficacy of microbes' CP production in vitro, while the amino acid composition of microbial proteins continued to be unchanged. The study concluded that SBP silage has specific effects on ruminal fermentation process, which may reduce feed intake but increase digestibility.

Through etherification, SBP cellulose can be turned into carboxymethyl cellulose (CMC). The carboxymethylation method was optimized in terms of the solution substrate, the amount of alkali, the amount of sodium chloro-acetate, the temperature, and the length of the reaction (Toğrul and Arslan, 2003). Carboxymethylcellulose is one of the most attractive derivatives Carboxymethylcellulose is one of the most attractive derivatives of cellulose (Toğrul and Arslan, 2003) Because of its surface characteristics, mechanical strength, tunable hydrophilicity, viscosity, accessibility, cheap manufacturing procedure, and excess of components. CMC may be widely used as food, paper, textile, biomedical engineering, pharmaceutical industry, wastewater treatment and energy production (Rahman et al., 2021). CMC may be produced in physical, chemical, and/or enzymatic process and lignocellulosic biomass to break down its structure and get cellulose. The quality of the cellulose depend on source material and extraction process (Pinto et al., 2022). Boguhn et al., (2010) noted that there are no significant impacts on milk production, milk protein, or fat content when high-yielding dairy heifers are fed up to 20% DM carboxymethyl cellulose.

Michel et al. (1988) The dehydrated cellulose SBP was pulverized and rinsed with 95% ethanol to produce a harmless and colorless substance that, after drying and sifting, could be utilized as dietary fiber. The chemical composition of SBP fiber was identical to that of the initial pulp: 26–32% cellulose, 21.5–23% hemicellulose, 22–24% uronic acids, 1–2% lignin, 7–8% protein, 7.5–12% ash, and 0.5% sucrose residual.

Ptak et al. (2021) The potential of utilizing SBP as feedstock for a variety of products with added value was reported, contributing to the implementation of a circular bio economy. The objectives include presenting the concept of a circular bio economy, characterizing the properties of sugar beet pulp (SBP), determining the production volume, and determining methodologies for deriving attractive biological products and energy sources that are renewable from sugar beet pulp. SBP can be used as a feedstock for various bio-products, making it a promising option for sugar industry.

Boudra et al. (2015) researched mycotoxins contents of SBP silage produced in different regions of France. Eight samples out of 40 (20%) were positive with mycophenolic acid and zearalenone being more prevalent; mycophenolic acid was detected in 5 / 40 samples, zearalenone was identified in 3 / 40 samples and ochratoxin A was detected in 1 / 40 samples.

Bodas et al. (2010) reported that inclusion of SBP to diets (the effects of 120 grams of SBP and 20 grams of $NaHCO_3$ per kg) decreased DM intake and daily weight gain, thus raising the ratio of feed to gain. SBP nutrition had no impact on carcass characteristics. In a separate study, Bodas et al. (2007) reported that partial additional of barley with SBP caused a high concentrations of acetate and pH and decrease in propionate and volatility fatty acids (VFA) in the rumen. Blood pH, bicarbonate, and packed cell volume parameters remained unaffected. In conclusion, SBP addition to cereal-based diets may improve ruminal condition and decrease ruminal acidosis risk.

Radchikov et al. (2016) plant waste, such as grain waste, SBP, molasses, alcohol bard, spent grains, pulp, and fruit and berry pomace, are valuable sources for the compound feed industry. The inclusion of cattle feed essence prepared from secondary SBP in the diet enhances digestive processes, improving nutrient digestibility by 1.8–7.3% and nitrogen deposition by 10.8–26.4%. Animals whose diets were supplemented by 20–25% (by weight) with the concentrate exhibited significant differences in blood total protein levels. Cows demonstrated the most pronounced tendency to reduce urea levels among study groups, replacing 20–25% of the diet (by weight) with 9.8 and 7.3%, indicating an activation of processes related to metabolism in the research groups.

Brand et al. (2009) carried out a study of 15 young Holstein Friesian bulls (18 months old) were fed wheat straw and in the treatment groups replaced with wet SBP at 4% and

8% on DM base. Feeding with SBP (4% SBP and 8% SBP) had lower dry matter intake and feed efficiency ratio compared to control group. The feeding of 8% SBP improved meat traits as general acceptance, juiciness, tenderness, beef flavor intensity, number of chews before swallowing, and Warner Bratzler Shear value. Chemical analysis was not differing among the groups. Similar findings were observed in another sheep experiment (Olfaz et al., 2005) and replacing of roughage 40 and 60 % with SBP to diets caused a decrease in dry matter consumption and DM conversion index compared to the control group. The consumption of a 60% sugar beet pulp diet resulted in an increase in the L* value of meat in rams. Conversely, the inclusion of 40% and 60% SBP in the diet led to a reduction in both final pH and cooking losses. There were no discernible sensory characteristics seen across the treatment groups. The inclusion of 60% sugar beet oil-based polyunsaturated fatty acids (PUFAs) resulted in an elevation in the levels of palmitic and linoleic acids in the meat, accompanied by a reduction in the concentrations of oleic, stearic, and arachidonic acids. The 40% SBP diet also raised the amount of linoleic acid, but it reduced the amount of stearic acid and arachidonic acid. The taste test couldn't tell the difference between the meats from control-fed and/or SBP-fed young pigs. So, 60% of SBP can be used to feed young Karayaka rams instead of grass hay without lowering the quality of their meat.

Clark and Armentano (1997) The beneficial effects of beet pulp neutral detergent fiber (NDF) as a supplement for alfalfa NDF was investigated in a study involving 16 mid-lactating Holstein heifers. Cows were fed a low forage, low fiber diet, a normal forage diet, and two low forage diets containing 5.3 g of NDF/100 g of DM from whole or ground desiccated sugar beet pulp. When beet pulp was substituted for alfalfa NDF, milk protein yield and concentration increased, whereas milk fat yield and concentration remained unchanged. The addition of beet pulp fiber to a low-forage, low-fiber base diet did not affect the amount of milk, protein, or fat the cows produced, but the proportion of protein in their milk was lower on the beet pulp diets than on the base diets. When the size of the beet blend particulates was reduced, DM intake increased, but milk production measurements remained unchanged. Changes in the lipid content of milk did not indicate that reducing the particle size of beet purée decreased its value as a source of fiber.

Kargar et al. (2021) examined the effects of replacing steam-flaked corn (SFC) in a starter diet with shredded SBP on selective intake, feeding and behavior, blood biochemical parameters, and growth of young Holstein dairy calves. A total 48 piglets and two dietary regimens containing either 0% or 25% SBP were used in the study. SBP increased intake of starter feed, total dry matter, crude protein, and neutral detergent fiber, while decreasing intake of starch and ether extract. The average daily gain, feed efficiency, final weight, and skeletal development did not change. The substitution of SBP had no effect on blood glucose, total protein, or albumin. However, calves fed SBP had a greater blood urea-N concentration and a lower albumin: globulin ratio than calves fed SFC. SBP was associated with increased meal duration, meal size, and ruminant frequency and duration, but decreased intervals between ruminations. The SBP-fed calves spent more time consuming, ruminating, and standing and less time reclining down and engaging in non-nutritive oral behaviors. In general, 25% SBP replacement had no effect on calf performance, but it increased time spent ruminating and consuming while decreasing non-nutritive oral behaviors.

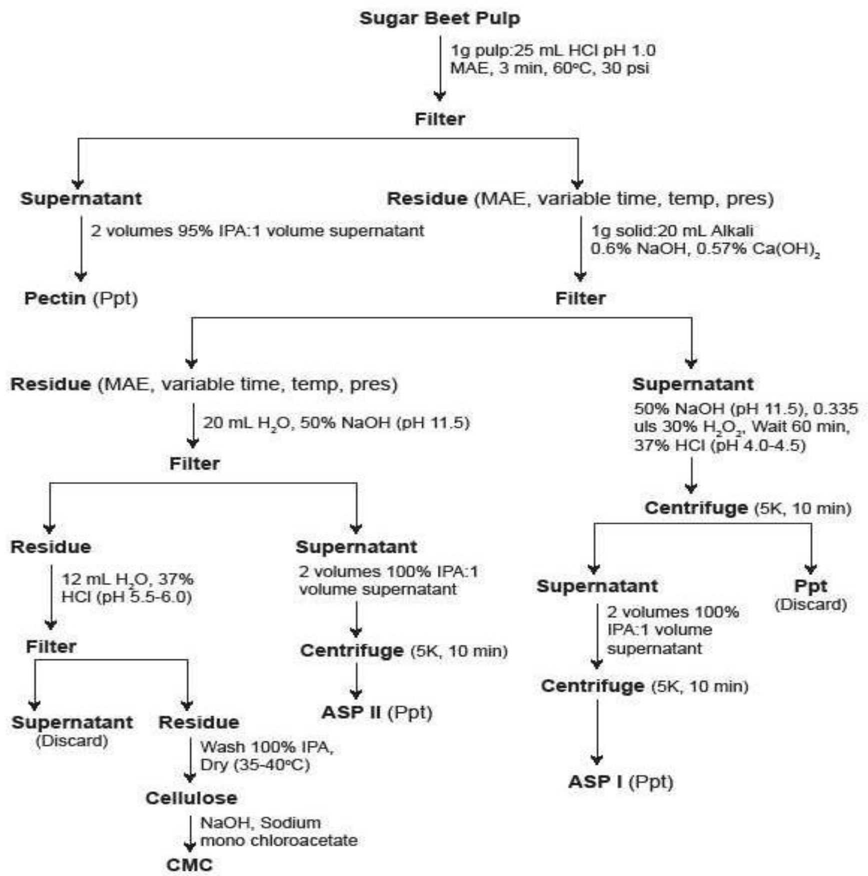


Figure 1. Production of pectin and carboxymethyl from SBP is showed (Hotchkiss et al., 2010).

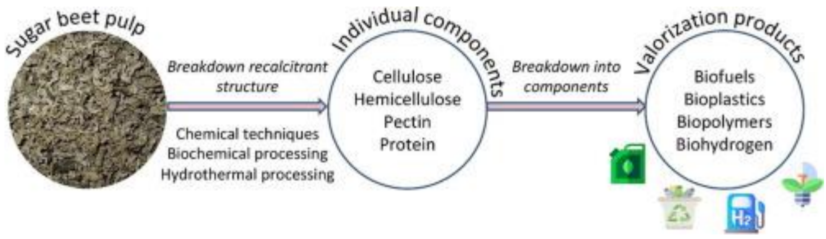


Figure 2. Production of different materials from sugar beet pulp (Usmani et al. 2022)

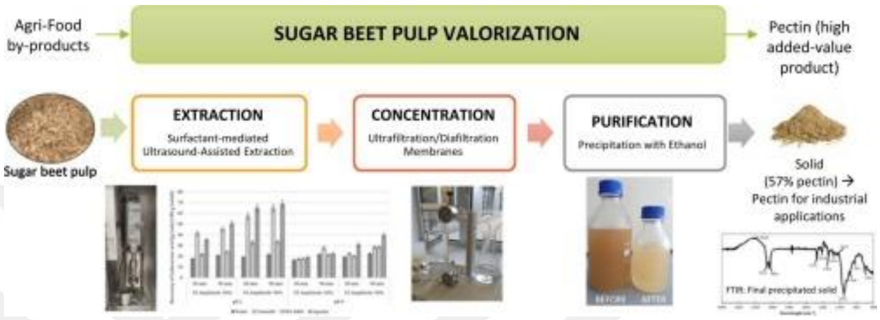


Figure 3. Sugar beet pulp valorization steps.

1.1. Pectin from sugar beet pulp

Pectin is a structural polysaccharide that can be found in all vascular plants (Willats et al., 2001) and is a natural part of a diet. Commercial pectin preparations are extracting from citrus peel, apple pomace, and waste from making juice. They are under use in food industry to prepare jams and jellies, candies, drinks, and acidified milk drinks and health benefits and packaging (Chandel et al., 2022). Also to obtain pectin SBP are using with Chemical extraction methods. The optimal HCl-extraction technique produced 19.53 % pectin to unprocessed SBP. Pectin had improved concentration of methoxy (20% degree of methylation). Neutral sugars mainly arabinose and galactose, and peak molecular weights ranging from 35,500 to 44,700 daltons. It exhibited a high water-retention capacity with a low viscosity (Phatak et al., 1988).

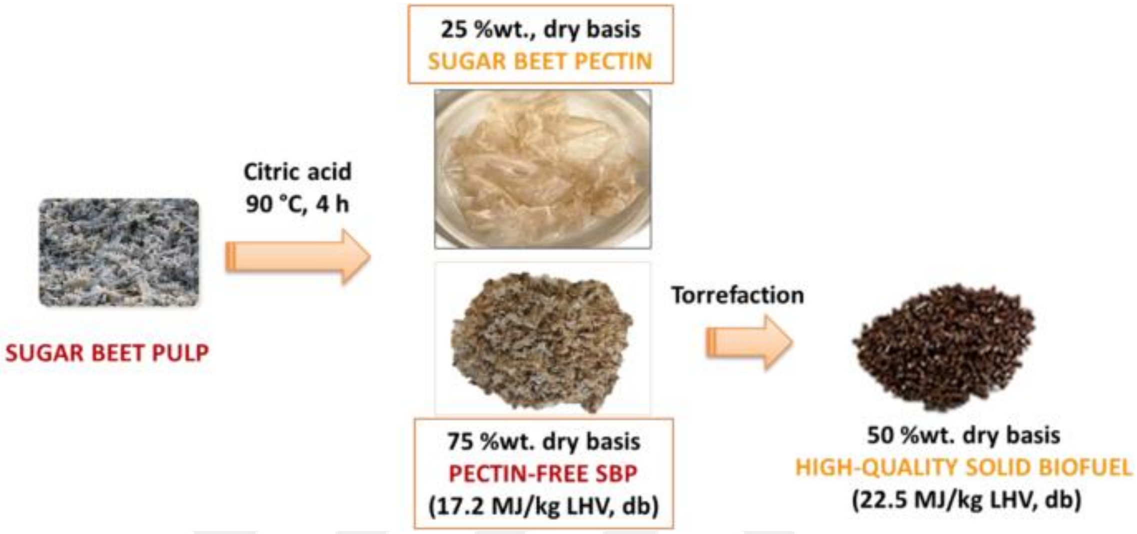
Pectin is a complex protein discovered in plant cell walls that is essential for plant growth, morphology, shape and protection. In addition to its use in food and specialty products as a gelling and stabilizing polymer, this substance has beneficial impacts on human well-being and medical use. At least 67 transferases are necessary for pectin bio synthesis, including glycosyl-, methyl-, and acetyltransferases. Studying the structure, role, and biosynthesis of pectin indicates that it performs a role in both the primary as well as the secondary cell walls. The modification of pectin biosynthesis is anticipated to have an effect on plant agronomic characteristics, such as biomass properties (Mohnen, 2008). Under optimal reaction conditions, a single pectin primarily constituted of homogalacturonan can be obtained, production it an ideal basic material for the produce of emulsified function (Ma et al. (2013).

Pectin is composed of three principal pectic polysaccharides: homoogalacturonan, rhamnogalacturonan-I, and rhamnogalacturonan-II. During biosynthesis, the pectic matrix's complexity of structure and heterogeneity are generated. Recent analytical techniques and anti-pectin devices are placing the structural complexity of pectin within the contexts of cell biology and development. The de-methyl-esterification of homogalacturonan in vivo by pectin methyl esterases is a crucial step in the modulation of local matrix properties. Rhamnogalacturonan-I is an assortment of spatially and evolutionarily controlled polymers, whereas rhamnogalacturonan-II is a highly conserved and stable pectic region (Willats et al., 2001).

Phatak et al. (1988) the yield and sugar composition of hemicelluloses and cellulose isolated from defatted, protein- and pectin-free, lignified, or delignified SBP were analyzed. The hemicelluloses derived from lignified SBP had a marginally greater yield of hemicelluloses (10.96%) than those obtained from delignified SBP (18.35%). The hemicelluloses' neutral sugar composition included glucose, galactose, arabinose, rhamnose, xylose, and mannose. Infrared spectra revealed a concentration band at 900 cm, representing the presence of some b-linked polysaccharides. Six additional phenolics were recognized in the combined product of alkaline nitrobenzene oxidation of lignin related with separated hemicelluloses and cellulose fractions.

Pectin is found sugar beet pulp, and the impact of organic acidity extractants on production and pectin characteristics is explored. The data indicated that the most optimal output occurred between pH 1.5 and 2 hours (Ma et al., 2013).

Huang et al. (2017) investigated the influence of various dehydration conditions on the properties of SBP pectin. All pectin samples are profoundly affected by the conditions of drying on their apparent viscosity and activation energy. Fourier transform infrared spectroscopy spectral analysis revealed that sugar beet pectin produced under varied dehydration conditions exhibited no significant structural differences. However, the zeta potential and conductivity of various desiccated sections varied from -47.9 to -55.6 mV and from 0.0079 to 0.0095 mS/cm, respectfully.



1.2. Cellulose from sugar beet pulp

The rumen is a complex digestive organ that efficiently digests plant fiber, particularly cellulose, through microbial fermentation. The rumen is home to anaerobic bacteria, fungi, and protozoa, which work together to break down the cellulose component. Although pure cellulose can be digested if the right conditions are met, but interactions between structural biopolymers in the matrix stop complete breakdown. Ruminal bacteria are the most active cellulose degraders, while fungi play a crucial role in disrupting recalcitrant tissue for degradative enzymes. Cellulose degradation in the ruminant is improved by microbial cellular connection and synergistic interactions with non-cellulolytic microorganisms, but prevented by variables resulting in lower ruminal pH (Weimer, 1992).

Sun and Hughes (1998) extracted four hemicelluloses and cellulose fractions from starch-based polysaccharides with different methods. The yield of hemicelluloses was slightly higher at 7.5% NaOH extraction, while cellulose yield was highest at 18.35%. Neutral sugar is composed up of glucose, arabinose, galactose, xylose, and small amounts of mannose and rhamnose. Infrared measurements presented a slowing up band at 900 cm1, which showed that there were some carbohydrates that were linked together. In the blend of alkaline nitrobenzene oxidation of lignin in the separate hemicelluloses and cellulose fractions, six more phenolics were found.

1.3. Diet fiber from sugar beet pulp

Cellulose is needed to ensure intestinal fullness and maintain its functions well in humans and animals. SBP has a good quality and highly soluble in the intestine fiber sources (Wróblewska et al., 2021). The decomposition of cellulose in SBP and its use as a source of human dietary fiber can distinguish it from normal sources of fibers. Various fiber sources that are not good for human feeding such as hay, hay, and vegetable by-products are used in the nutrition of animals.

Ruminant animals need physically effective fibre, specifically NDF, to enhance chewing and ruminal activity. This fibre content was related to particles size and can enhance salivary flow, the ratio of acetic to propionate, which milk levels of lipids, and rumen pH. Ensuring a suitable level of NDF and size of particles in the diet of ruminants increases the amount of dry matter intake and digestion, positively impacting ruminant performance. The National Research Council recommends maintaining NDF content at 25% of dietary dry matter, with at least 75% from forage. However, no recommendations for physically effective fibre are currently available due to a lack of standards and validated data (Banakar et al., 2018).

Pérez-Barbería et al. (2004) examined in fibre digestibility in ruminants across various feeds with varying fibre to lignin ratios. The effects showed that thoroughly related species have similar fibre digestibility values compared to distant species. The body mass had no significant influence on the digestibility of fibers. In a species' normal diet, the digestibility of fiber is estimated to increase with grass and decrease with forage. The evolutionary model inferred that fibre digestibility evolution accelerated over time, possibly due to cooperation amongst cattle and adaptability to high-fiber diets.

1.4. Global warming and contributing effects of animals

Recently global warming and animals effect on it is main topic in scientific meetings and there are big debates to increase greenhouse gases in the earth. However, last a few decades, due to global warming was caused by CO_2 and other greenhouse gas (GHG) emissions, according to environmental experts worldwide. Some animal and plant species have already gone extinct, and if global warming continues, more will as climate change gets more severe (Philander, 2012). Greenhouse gases (GHG) are produced by both natural and human activities. Animal agriculture accounts for 18% of the greenhouse gases that contribute to global climate. Reducing greenhouse gas (GHG) emissions from agriculture, particularly from rearing cattle, is a top priority because it could significantly reduce global warming. Included among the ruminant species are cows, horses, sheep, and goats (Sejian and Naqvi, 2012). The agricultural sector contributes for 25.5% of global emissions of greenhouse gases, with methane having 21 times more warming potential than carbon dioxide. Ruminant livestock contributes significantly to methane emissions, causing environmental problems and energy losses. To reduce methane emissions are making some modification on dietary, genetic selection and rumen microbioata, vaccines against methanogenic bacteria (Moumen et al., 2016).

Global warming has both long-term and short-term effects. GHG emissions ware made up of six gases: carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), sulfur hexafluoride (SF_6), hydrofluorocarbon (HFC), and perfluorocarbon (PFC) (Haradhan, 2011). The global demand for animal production is projected to double by 2050 as a result of rising living standards. Climate change is a hazard to animal production because it affects feed crop quality, access to water, animal and milk production, maladies, reproduction, and biodiversity. The production of livestock is constrained by rising animal water consumption, rising agricultural land demand, and food security issues. The livestock industry is responsible for 14.5% of global emissions of greenhouse gasses, contributing to the acceleration of climate change. To transition to sustainable production of livestock, it is essential to evaluate mitigation and adaptation strategies tailored to the area and livestock production system, as well as implement policies that encourage and promote adaptation and mitigation to climate change (Rojas-Downing et al., 2017).

Agriculture's methane emissions are caused by the decomposition of manure and the fermentation of bacteria in the rumen. In order to minimize methane emissions, research suggests extended rumination times, an increase in propionate, and a decrease in acetic and butyrate in the rumen, as well as a higher-energy diet. Asparagopsis taxiformis contains bioactive bromoforms that inhibit methane production. Additionally, plant extracts, lipid supplements, and low-CH4 cows can aid in increase methane emissions. Further research is required to comprehend the impact of methanogenesis inhibition on rumen fermentation and post-absorptive metabolic processes, thereby enhancing the output and efficiency of livestock (Bačėninaitė et al., 2022).

In the next decades, atmospheric CO_2 will double, raising global temperatures by 1.4°C-5.8°C. Human actions cause this rise. High temperatures stimulate metabolic processes like ROS and OS in animals. This causes aberrant physiology and oxidative stress, reducing survival and lifespan. Global warming may cause OS, decreased survivorship, and lifespan in livestock, especially poikilotherms. In homeotherms, altered O2 consumption at increased temperatures may raise OS risk. Biologists must understand how global warming affects animal lifespan in the next millennium (Paital, 2016).

The average agricultural emissions in the EU-15 are estimated to be 10.2 million metric tons, with two-thirds originating from enteric fermentation and one-third from animal manure. Methane production in the rumen is affected by the molar proportion of volatile fatty acids generated during fermentation. Alternative methods for reducing methane include the development of anti-methanogenic compounds, the introduction of alternative electron acceptors in the rumen, and the reduction of protozoal populations. Due to the high fiber content of ruminants, which can be transformed into high-quality sources of protein such as meat and milk, ruminants are crucial to human survival (Moss et al., 2000).

Ruminant animals produce greenhouse gas methane, which causes global warming. Methane is generated in the ruminant as a hydrogen waste product. To reduce methane emissions, rumen microbes, compounds, antibiotics, and organic materials like lipids, fatty acids, and herbal extracts are manipulated. Among the most recent methods are vaccines against methanogenic microorganisms. Due to their efficacy and affordability, ionophore antibiotics are extensively used, but natural alternatives are gaining popularity due to health concerns. Potential methane inhibitors include natural substances that improve propionate, an alternative hydrogen absorber to methane (Kobayashr, 2010).

Methane emissions may be reduced by longer rumination, more propionate production, and a higher-energy diet. Bioactive bromoforms from Asparagopsis taxiformis may lower intestinal methane emissions in animals. Methane emissions may be reduced by essential oils, tannins, saponins, and flavonoids. Emissions may be reduced using responsible plant extracts. Reduce greenhouse gas emissions by 20% with minimum lipid addition and low-CH4 cows. Suppressing methanogenesis in rumen fermentation and post-absorptive metabolism may boost animal output (Bačėninaitė et al., 2022).

A recent study predicts that temperature extremes will increase in frequency, intensity, and length, with variance changes affecting species more than mean temperature changes. The study combines fine-grained climate predictions for the years 2050–2059 with data on how well 38 species of ectothermic invertebrates endure temperature changes. The results show that projections based on changes to the average temperature are very different from those that include changes to both the mean and the average temperature. Performance decreases are more likely in temperate species, emphasizing the need for fine-grained temporal data to measure and project performance (Vasseur et al., 2014)

To increase livestock production, it is essential to reduce greenhouse gas emissions and climate change's effects. Controlling rumen microbial ecosystems, plant secondary metabolites, ration balancing, and manure management are examples of mitigation strategies. Adapting to climate change may necessitate substantial modifications to production technologies and agricultural systems. Direct effects include performance, milk output, reproduction, metabolic activity, and the incidence of disease infection. Reducing the availability of water, pasture, and fodder resources are indirect effects. To mitigate environmental stress on livestock, multidisciplinary methods focusing on feeding, shelter, and animal hygiene are required (Naqvi and Sejian, 2011). The researchers suggest extended rumination times, a rise in propionate and a drop in acetate and butyrate in the rumen as well as a higher-energy diet. Plant extracts, lipid supplements, and low-CH4 cows can aid in decrease methane emissions. Asparagopsis taxiformis contains bioactive bromoforms that inhibit methane production (Sallam et al., 2007).

Recent research on nutritional assessment utilizing in-vitro techniques has garnered popularity due to its simplicity of adoption, consistency, and reduced animal use... These techniques have potential applications in new areas of ruminant nutritional research (Getachew et al., 2005). So, in this study, SBP and its products evaluated in cow diets in terms of chemical composition, gas production and *in-vitro* digestibility.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

The dry sugar beet pulp and its products (cellulose, diet fiber, carboxyl methyl cellulose (CMC) and pectin) were obtained from Kayseri Şeker Factory located in Kayseri, Turkiye.

2.2. Methods

2.2.1. Preparation of samples

The samples used in the study were grounded to 1 mm in diameter and packed in numbered nylon.

2.2.2. Chemical analysis

To determine chemical analyses, Weende analyses and ADF and NDF analyses were done in the University of Erciyes, Faculty of Agriculture Feeds and Department of Animal Sciences at the Feed Science and Animal Nutrition Laboratory.

2.2.2.1. Determination of dry matter

Wet SBP was dried 75 °C for 72 hours. Cellulose, diet fiber, CMC, and pectin were subjected to a dry in the oven at 75 °C for 72 hours. Dry matter content were calculated according to this formula.

Dry matter=sample last weight/sample first weight ×100

2.2.2.2. Determination of crude ash

About 0, 5 g the SBP, cellulose, diet fiber, CMC, and pectin samples were subjected at 550 °C for 5 hours and the amount of ash formed from the remaining inorganic materials was expressed in%.

$\% \text{ Ash} = ((W_3 - W_1) / (W_2 - W_1)) \times 100$

Where;

W1=Initial weight of bag

W2 = Initial weight of bag + weight of the sample

W3= Initial weight bag + weight of the dried sample

2.2.2.3. Determination of crude oil

Ratios of the feed samples were determined by extraction with the Velp fat extractor (Model 148, Italy) with petroleum ether.

Picture 2.1. Crude fat analyze machine

2.2.2.4. Determination of crude protein

The determine protein content of feeds the Dumas nitrogen analyzer (NDA 701, VELP Scientific Ltd. Co. Italia) was used. An average weight of 0.040 mg samples placed in a specific aluminum sheet before being placed in the device assigned location.

Picture 2.2. Protein analyze machine

2.2.2.5. Determination of acid detergent fiber (ADF)

To determine acid detergent fiber content of feeds the Ankom fiber analyzer (ANKOM 2000 Automated Fiber Analyzer - ANKOM Technology, USA) were used according to its catalogue. For method, 1.800 of distilled water + 40 ml acid detergent powder + 54.50 sulphuric acid waited for 1hour and half, after that we put petroleum benzene to acetone 10m to dry then we put heated machine degree 105℃ into 2h then we measured samples.% ADF (as-received basis) = (W3 - (W1 x C1)) x 100 W2.

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)

Picture 2.3. ADF and NDF analyze machine

2.2.2.6. Determination of neutral detergent fiber (NDF)

To determine neutral detergent fiber content of feeds the Ankom fiber analyzer (ANKOM 2000 Automated Fiber Analyzer - ANKOM Technology, USA). To prepare solution, 1.800 of distilled water +120 gram Neutral detergent solution powder+20 ml trithyline glycol + Sulphide 20 gram + alpha amylose 4ml + alpha amylose 8 ml of side machine and waited for 1hour and half, after that we put petroleum benzene to acetone 10m to dry then we put heated machine degree 105℃ into 2h then we measured samples.

To calculate NDF % =(weight of crucible+weight of residue)–weight of crucible/weight of sample x 100. Neutral detergent solubles: NDS % = 100–NDF%.

2.2.2.7. Conducting the in vitro trial

The pistons of the measuring cylinder (Figure 3.4) were lubricated with silicone and inserted within the measuring cylinder; 24 samples were generated. Five groups, including sugar beet pulp, cellulose, diet fiber, CMC, and pectin, were added to a cow's diet in order to calculate the amount of gas produced, and the gas readings were then adjusted in this manner. The rumen fluids obtained from the rumens of animals slaughtered at a commercial slaughterhouse in the region, whose feeding and disease histories are known, were transported to the laboratory in a thermos, filtered with a four-fold strainer, and mixed with artificial saliva in a ratio of 2:1 to create fermentation liquid. During incubation, the obtained mixture was transferred into preheated 30 ml syringes and stored in a 39°C water bath. All liquids were subjected to a 24-hour incubation period, and the gas production amounts were recorded. Due to the lack of significant differences between repetitions in the groups, the groups were not reanalyzed in the obtained results.

For the determination of in vitro gas production, the Hohenheimer Butterwort Test (HFT) or Hohenheim feed test was utilized (DLG., 1981).This method was developed at Hohenheim University in Germany. This technique is based on the measurement of gas production (CO_2 and CH_4) resulting from a 24-hour incubation of nutrients with rumen fluid in an incubator. The obtained results are used to determine the digestibility of organic matter and the net energy lactation (NEL) content of feedstuffs. The relationship between gas production and in vitro digestibility is also determined.

Measuring cylinder (36 mm diameter, 200 mm long) with a 100 ml volume marked and a 50 mm long and 5 mm diameter silicone tube attached to the thin end, a clamp that closes the measuring cylinder on the tube, device for taking rumen fluid. Dispenser (50 ml piston and adjustable to 30 ml volume), it would bottle. Magnetic stirrer, water bath (set at $39^{\circ}C$), and carbon dioxide tube.

Picture 2.4. Measuring Cylinder

To carry out the procedure those solutions were used.

Macro mineral solution: 5.7 g Na_2HPO_4 + 6.2 g KH_2PO_4 + 0.6 g $MgSO_4$ $7H_2O$ + It is dissolved in pure water and complete to 1000 ml.

Micro mineral solution: 13.2 g $CaCl_2$ $2H_2O$ + 10 g $MnCl_2$ $4H_2O$ + 1.0 g $CoCl_2$ $6H_2O$ + 8.0 g $FeCl_3$ 6 H_2O + It is dissolved in pure water and complete to 1000 ml.

Buffer solution: 39 g Na HCO_3 + 4 g (NH_4) HCO_3 + It is dissolved in pure water and completed to 1000 ml.

Reassuring solution: 100 mg dissolve reassuring in pure water and makeup to 100 ml.

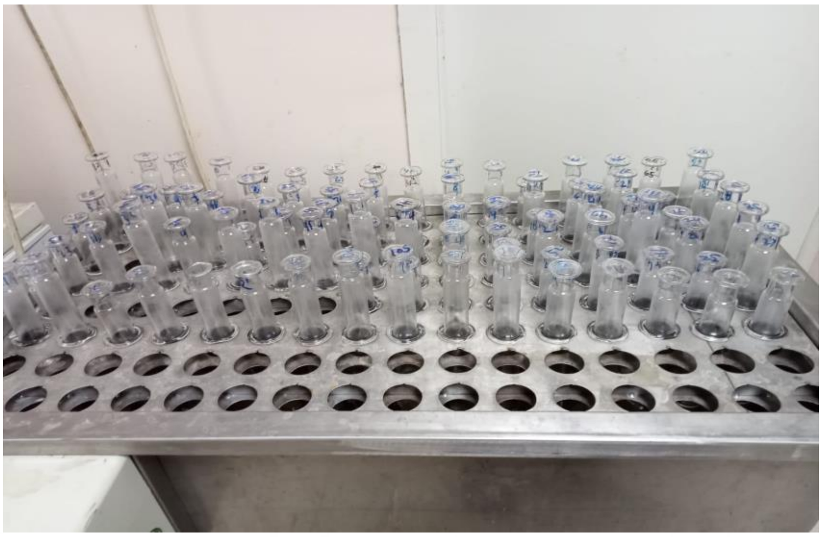
Reduction solution: It is prepared fresh in every work. First 1.99 ml of 1 N Noah solution is put into 47.45 ml of distilled water, 285 mg of Na2S 7HO is added.

The solutions prepared as described above were put into bottle in the amount and order given below.

474.50 ml distilled water + 0.12 ml micro mineral solution + 237.23 ml buffer solution + 237.23 ml macro mineral solution + 1.22 ml resazurin solution + 49.44 ml reduction solution. This mixture was prepared just before the rumen fluid was taken (DLG., 1981), mixed with a magnetic stirrer in a $39^{℃}$ water bath under CO_2 gas, and kept until rumen fluid was added.

Rumen fluid was obtained from slaughtered cows that know their feeding and disease history and sent to slaughter. The rumen fluid was immediately taken to the laboratory after it was placed in a thermos to maintain its temperature. In the laboratory, 500 ml of rumen fluid (1/2 ratio of the mixture) was added to the mixture prepared in the woulf bottle. CO_2 gas was continuously injected into this mixture with a tube, while the color change was checked for about 15 minutes. After putting 30 ml of rumen liquid mixture with the dispenser into the measuring cylinders where the sugar beet pulp and products and pectin added dairy cattle diets. The cylinders were placed in the incubation cabinet as shown in Picture 3.5. Incubation took place at $39^{℃}$ for 24-hours. Each example was studied in 3 repetitions. The liquids prepared for all treatments were subjected to 24-hour incubation time and gas production amounts were recorded period.

After collect gas in cylinders and gases were injected to methane detector and got results, and determined in % total gases.



Picture 2.5. Incubation of prepared rumen fluids

Picture 2.6. Methane detector

2.2.2.8. Calculation of OMD and ME content of samples

After the gas production amounts of plant by-products were determined, OMD (%), ME (MJ / kg), and NEL (MJ / kg) values were calculated using the equations reported by (Menke and Steingass, 1988) and shown below using the equations below.

For forage feeds, ME (MJ/kg DM) = 2.20 + 0.136GP + 0.057CP + 0.0029CF2

For concentrate feeds, ME (MJ/kg DM) = 1.06 + 0.157GP + 0.084CP + 0.22CF − 0.081CA

$$NEL(MJ/kg\ KM) = 0.115 \times GP + 0.0054 \times HP + 0.014 \times HY - 0.0054 \times HK - 0.36$$

$$OMD\ (\%\ KM) = 0.9991 \times GP + 0.0595 \times HP + 0.0181 \times HK + 9.00$$

Organic matter was calculated difference between dry matter and crude ash%.

Organic matter= (dry matter-crude ash)

2.2.2.9. Statistical analyses of data

To evaluate the data SPSS 22.0 program (IBM, USA) was used to show the differences among the treatments via using One-way Anova procedure. To separate differences Duncan test was applied for averages. For significance $P<0.05$ was considered.

CHAPTER 3

RESULTS

In this thesis two experiments were carried out. One of them was related to evaluation of dry SBP and its product's (diet fibre, cellulose, CMC and pectin) chemical composition, total gas production and methane production in beef cattle. Another one was related to pectin addition to dairy cow diets and its effect on chemical composition, total gas production and methane production.

Experiment - I

3.1. Evaluation of SBP and its product's (diet fibre, cellulose, CMC and pectin) chemical composition, gas production, digestibility and energy values in beef cattle

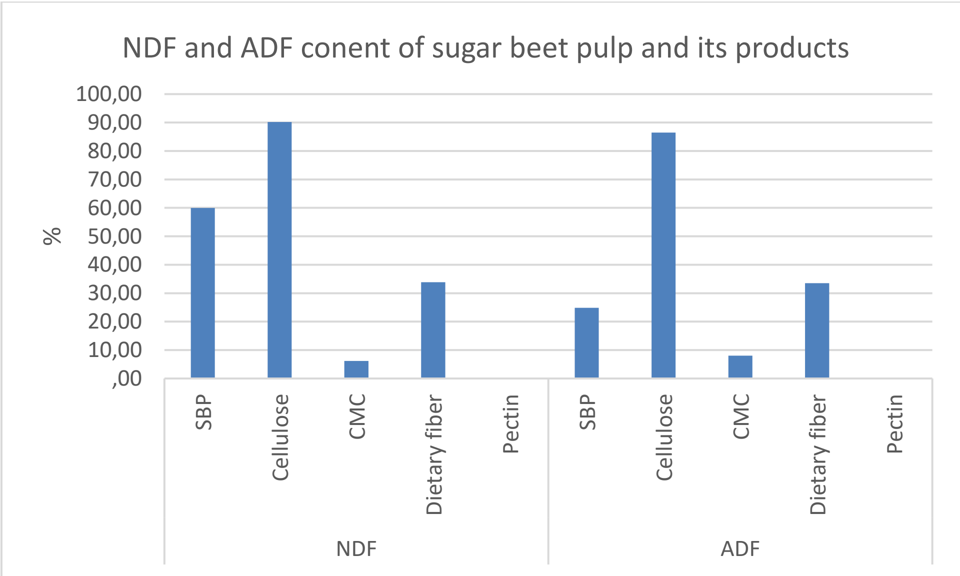
3.1.1. Chemical Composition of SBP and its Product's (Diet Fibre, Cellulose, CMC and Pectin)

The dry matter, crude protein, crude oil, crude ash, ADF, NDF and crude cellulose analyzes of SBP and products are shown in table 3.1. There were no significant differences between the SBP and its product's dry matter content. These products already dried before and stored therefore it may be possible results. SBP, diet fiber and cellulose product's protein content was higher than that of pectin and CMC and also CMC was lower than pectin ($P<0.01$). In the CMC product, protein did not found. The crude oil content of diet fiber was higher than the other products and also SBP's oil content was higher than those of cellulose, pectin and CMC product ($P<0.01$). CMC product crude oil content was lowest. In the cellulose product's crude ash content was higher than those of other products. Crude ash of pectin was higher than that of other products except cellulose product ($P<0.01$). In cellulose product the NDF content was higher than other products and SBP's NDF content was higher than the CMC and pectin products. The cellulose product's ADF content was higher than others and the diet fiber was higher than SBP (P<0.01). In the pectin product ADF and NDF did not determined. There were no differences among the products in terms of organic matter content.

Table 3.1. The dry matter, crude protein, crude oil, crude ash, ADF, NDF and crude cellulose analyzes of SBP and products

Item	Chemical composition, %						
	Dry matter	Crude protein	Crude oil	Crude ash	NDF	ADF	Organic matter
SBP	89.78±1.67	8.63±0.20a	0.56±0.00b	4.59±0.27c	59.96±0.87b	24.84±0.16c	85.19±01.43
Cellulose	88.99±3.13	7.60±0.69a	0.47±0.01c	8.54±0.07a	90.20±0.41a	86.47±0.34a	80.45±03.20
CMC	87.39±0.33	0.00±0.00c	0.23±0.01d	4.24±0.00c	6.18±0.00d	8.01±0.00d	83.16±0.34
Diet fiber	88.35±2.72	7.65±0.49a	0.68±0.02a	4.24±0.02c	33.87±0.74bc	33.53±0.47b	84.12±02.74
Pectin	86.26±0.57	5.75±0.10b	0.43±0.01c	7.97±0.07b	0.00±0.00d	0.00±0.00d	78.29±0.58
Average	88.16±0.83	5.93±0.84	0.47±0.04	5.91±0.52	36.8±9.34	30.57±8.47	82.24±01.02
P	0.762	0.001**	0.001**	0.001**	0.001**	0.001**	0.175

[a,b,c,d]: Differences between the averages are significant in the same column with different letter, SBP: sugar beet pulp, CMC: carbohydroxymethyl cellulose, NDF: Neutral detergent cellulose, ADF: Acid detergent cellulose, ADL: Acid detergent lignin, P: probability, **: significant at P<0.01.



Graphic 3.1. Effects of pectin addition on chemical composition of diets

3.1.2. Gas production value of SBP and its product's (diet fibre, cellulose, CMC and pectin) in beef cattle

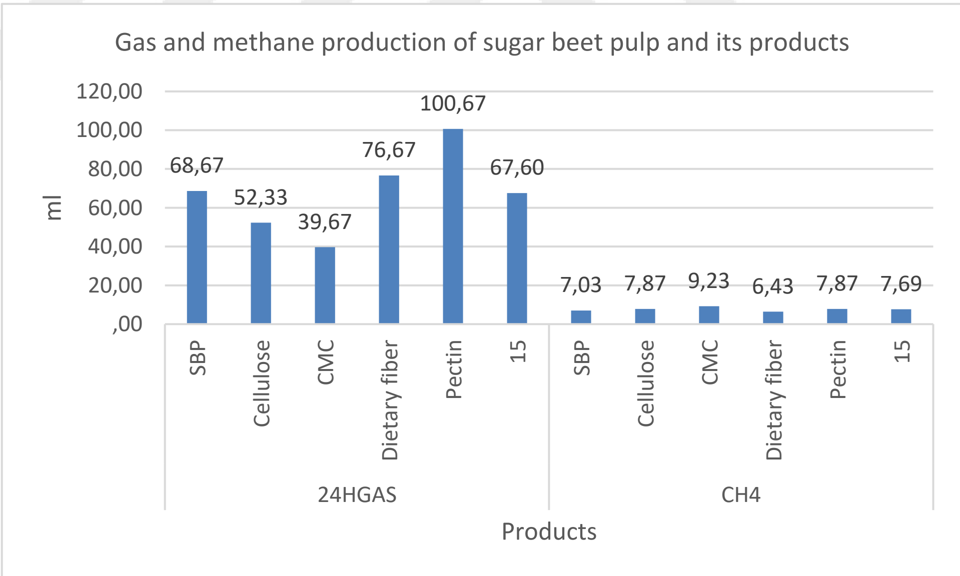
Total gas production potential, CH4 gas production ratio of SBP and its products are given in Table 3.2. Gas production in pectin at 6, and 12 hours was significantly higher than other products ($P<0.01$). SBP and other products were similar in gas production value at 6 and 12 h period. However, SBP and dietary fiber and pectin products total gas production at 24-hour were higher cellulose and CMC products ($P<0.01$).

There were no significant differences among the SBP and its products in terms of methane production ratio. However, pectin product methane production numerically was higher than other when compared to other products.

Table 3.2. Gas production in 6, 12 and 24 h and methane production of SBP and its products

Item	Gas production, ml 6 h	12 h	24 h	Methane production, %
SBP	11.50±2.12^{b}	19.00±12.17^{b}	49.00±18.52^{ab}	7.03±0.86
Cellulose	7.00±7.07^{b}	11.67±6.66^{b}	13.33±14.74^{c}	7.87±0.96
CMC	2.50±0.71^{b}	5.33±0.58b	9.67±0.58c	9.23±0.26
Dietary fiber	8.33±1.53^{b}	17.33±6.51^{b}	40.33±17.79^{b}	6.43±0.19
Pectin	41.00±16.52^{a}	62.67±8.33a	70.67±5.13^{a}	7.87±0.37
Average	15.83±17.12	23.20±22.03	36.60±26.17	7.69±0.34
P	0.011**	0.001**	0.001**	0.076

SBP: sugar beet pulp, CMC: carbohydroxymethyl cellulose, ^{a,b,c,d}: Differences between the averages are significant in the same column with different letter, P: probability, *: significant at P<0.05, **: **significant at P<0.01.



Graphic 3.2. Gas and methane production of sugar beet pulp and its products

3.1.3. Dry and organic matter digestibility and energy values of SBP and its product's (diet fibre, cellulose, cmc and pectin) for dairy cows

Average of dry and organic matter digestibility, and metabolic (ME, MJ and kcal/kg and net lactation (NEL) energy values of SBP and products is presented in Table 3.3.

The average SBP and products of dry matter digestibility (DMD) showed that pectin had a higher value than other products, while DMD of diet fibre was higher than SBP, cellulose; DMD of SBP was higher than those of CMC and cellulose; DMD of CMC was higher than CMC (P <0.01).

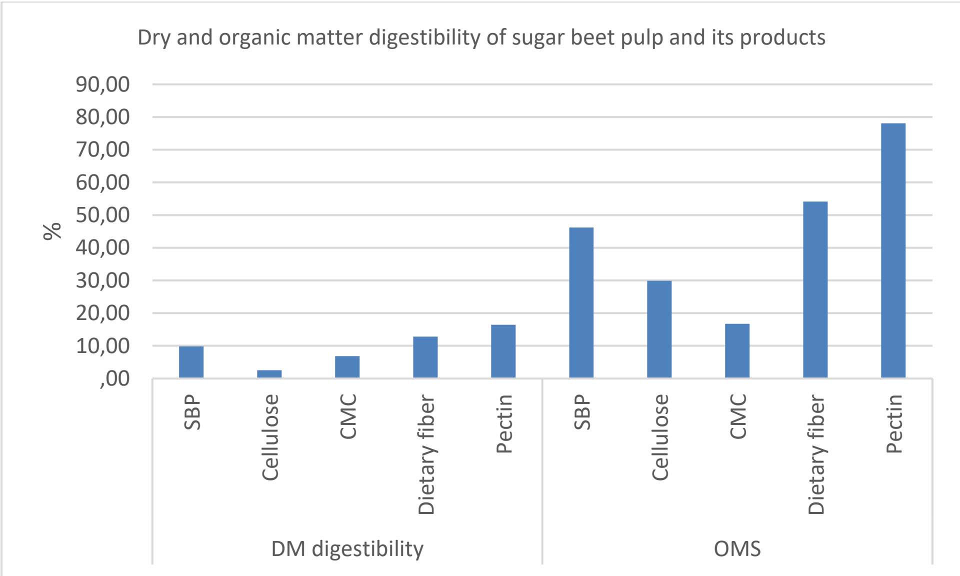
OMD values of SBP, diet fibre and pectin were higher than cellulose and CMC; OMD value of Cellulose was higher than CMC product (P<0.01).

ME (MJ/kg), NEL and ME (kcal/kg) values of SBP, diet fibre and pectin were higher than cellulose and CMC products (P<0.01).

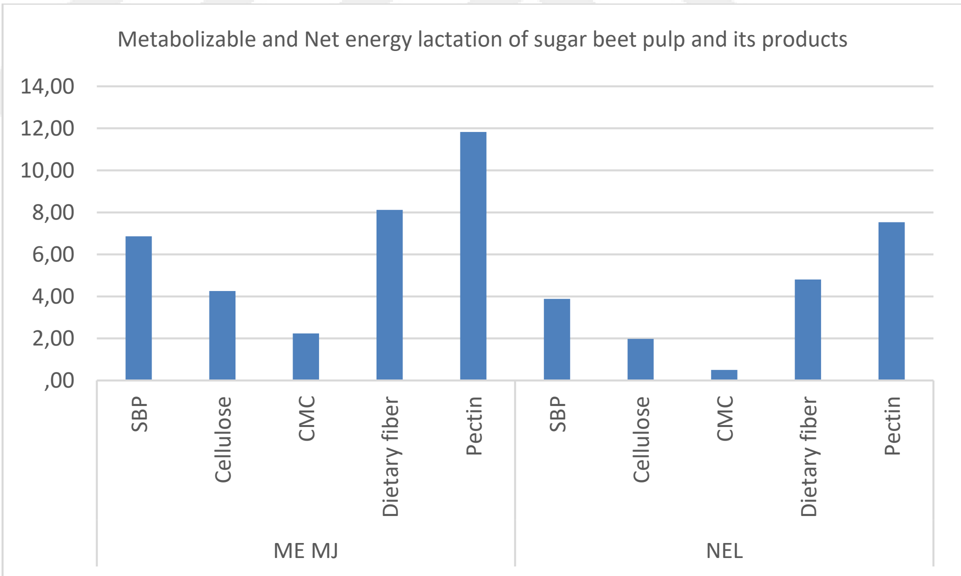
Table 3.3. Average of dry and organic matter digestibility, and metabolic (ME, MJ and kcal/kg and net lactation (NEL) energy values of SBP and products

Item	DMD, %	OMD%	ME, MJ/kg KM	NEL, MJ/kg KM	ME (kcal/kg)
SBP	9.85±0.63[c]	46.18±6.32[a]	6.86±0.99[a]	3.88±0.73[a]	1639.0±237.9[a]
Cellulose	2.51±01.18[e]	29.94±4.35[b]	4.26±0.68[b]	1.98±0.50[b]	1018.3±163.2[b]
CMC	6.85±0.38[d]	16.74±0.33[c]	2.24±0.05[b]	0.50±0.04[b]	534.0±12.50[b]
Diet fibre	12.83±0.51[b]	54.21±4.09[a]	8.12±0.64a	4.81±0.47[a]	1942.3±153.6[a]
Pectin	16.45±0.15[a]	58.75±3.15[a]	8.81±0.51[a]	5.31±0.37[a]	2105.7±122.5[a]
Average	9.70±01.31	41.16±4.47	6.06±0.70	3.30±0.51	1447.9±168.1
P	0.001**	0.001**	0.001**	0.001**	0.001**

[a,b,c,d]: Differences between the averages are significant in the same column with different letter, SBP: sugar beet pulp, CMC: carbohydroxymethyl cellulose, GP: gas production DMD: dry matter digestibility, OMD: organic matter digestibility, ME: metabolic energy, NEL: net energy lactation, MJ: Mega joule, P: probability, **significant at P<0.01.



Graphic 3.3. Dry and organic matter digestibility of sugar beet pulp and its products



Graphic 3.4. Metabolizable and Net energy lactation of sugar beet pulp and its products

Experiment – 2

In this experiment, effects of pectin from SBP added to dairy cow diet and evaluated its effect on chemical composition, total gas production, methane production (% of total gas) and energy values for dairy cattles. The obtained results were presented in below.

3.2. Effects of Addition of Pectin to Dairy Cow Diets on Chemical Composition, Gas Production and Energy Values

3.2.1. Effects of Addition of Pectin to Dairy Cow Diets on Chemical Composition

The dry matter, crude protein, crude fat, crude ash, ADF, NDF, crude cellulose analyses and organic matter of cow diets with added pectin are shown in Table 3.4. Dry matter content of control and pectin 0.5% diets were higher than that of pectin 1% added diets ($P<0.01$). In crude protein, there were no significant differences among the control, pectin 0.5%, and pectin 1% products.

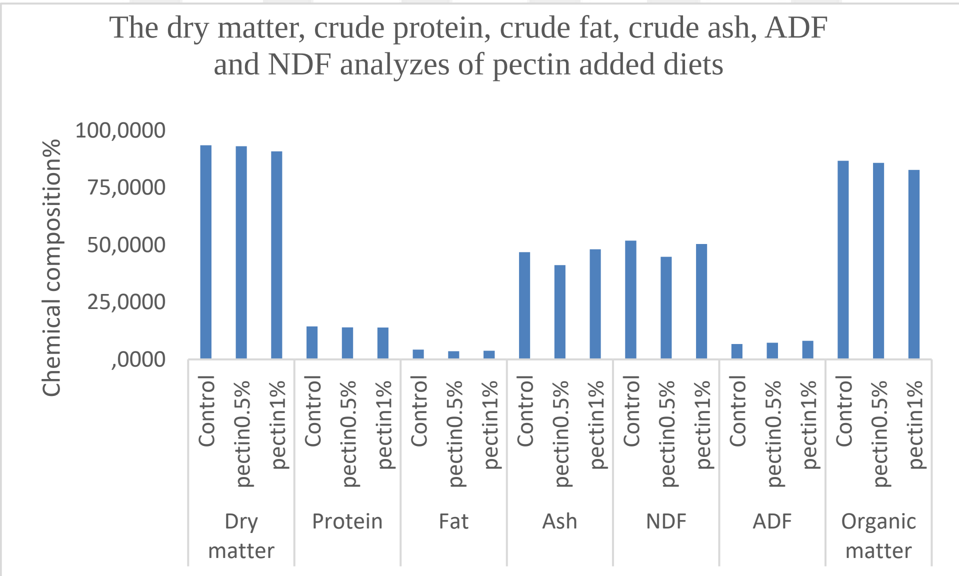
Control diet's, fat and ADF content was significantly higher than that of other products pectin 0.5% and pectin 1% added diets.

Crude ash content of control diet was lower than other product pectin 0.5 added diet's crude ash content was lower than a pectin 1% added diet ($P<0.01$). Control is lowest in ash content ($P<0.05$). In the pectin1% product the NDF content was higher than control and pectin 0.5% was lowest. The control product ADF content was higher than others and the pectin 1% was higher than pectin 0.5 % and only pectin ($P<0.01$). There were no differences among the products in terms of organic matter content. Organic matter content in Control and Pectin 0.5 products were higher than and pectin 1% added products and pectin 1% products ($P<0,01$).

Table 3.4. The dry matter, crude protein, crude fat, crude ash, ADF and NDF analyzes of control and pectin with diet products

Item	Chemical composition, %						
	Dry matter	Protein	Crude fat	Crude ash	NDF	ADF	Organic matter
Control	93.53±0.47[a]	14.38±0.04	4.24±0.13[a]	6.72±0.09c	46.86±0.34[b]	51.89±0.40[a]	86.80=0.97[a]
Pectin 0.5%	93.11±0.56[a]	13.96±0.21	3.58±0.17[b]	7.27±0.17b	41.21±0.32[c]	44.84±0.27[c]	85.85±0.30[a]
Pectin 1%	90.91±0.40[b]	13.91±0.10	3.80±0.04[b]	8.09±0.07a	48.12±0.30[a]	50.39±0.09[b]	82.82±0.38[b]
Average	92.52±0.44	14.08±0.10	3.87±0.12	7.36±0.21	45.40±01.07	49.04±01.08	85.16±0.63
P	0.004**	0.088	0.025*	0.001*	0.001**	0.001**	0.001**

[a,b,c,d]: Differences between the averages are significant in the same column with different letter, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin, P: probability, *: significant at P<0.05, **:significant at P<0.01.



Graphic 3.5. The dry matter, crude protein, crude fat, crude ash, ADF and NDF analyzes of pectin added diets.

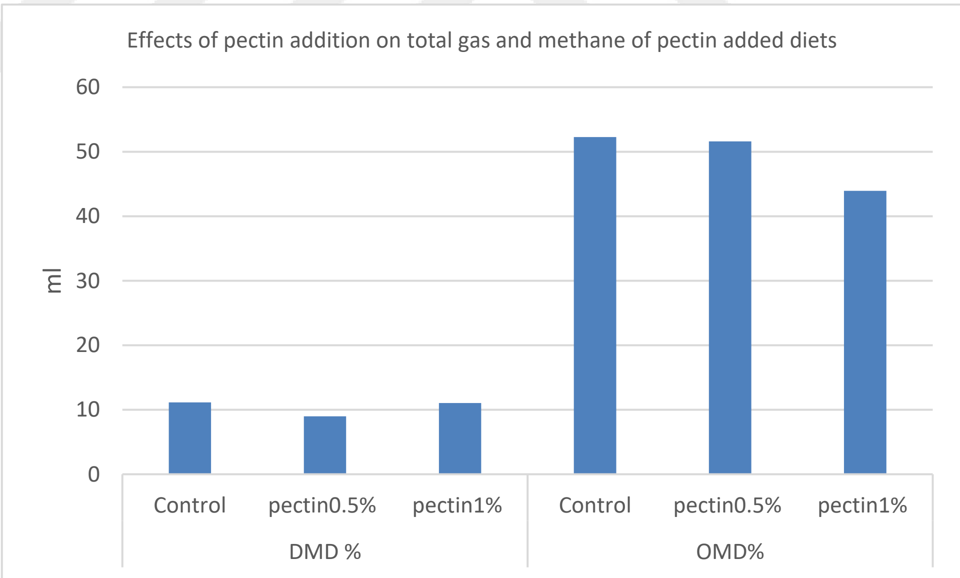
3.2.2. Effects of addition of pectin to dairy cattle diets on gas production (GP)

The total gas production and CH4 ratios of pectin and cow diets are given in Table 3.5. The products in terms of gas production in pectin 1% at 6 hours were higher than the control, and pectin 0.5 product added product was similar to others (P<0.01) at 12 hours, 24 hours, and methane production ratio were no significant differences among the control, pectin 0.5%, and pectin 1% products.

Table 3.5. Effects of pectin addition to dairy cattle diets on total gas and methane production

	Gas production, ml			Methane gas
Item	6h	12h	24h	production, %
Control	14.67±01.45b	30.00±02.65	42.33±3.48	10.33±03.48
Pectin 0.5%	13.00±02.00b	27.67±03.38	41.67±3.76	9.67±03.76
Pectin1%	23.00±03.21a	26.33±04.70	34.00±3.06	2.00±03.06
Average	16.89±01.94	28.00±01.91	39.33±2.18	7.33±02.18
P	0.048*	0.783	0.243	0.282

[a,b,c,d]: Differences between the averages are significant in the same column with different letter, DM: dry matter, P: probability, *: significant at P<0.05.



Graphic 3.6. Effects of pectin addition on total gas and methane of pectin added diets

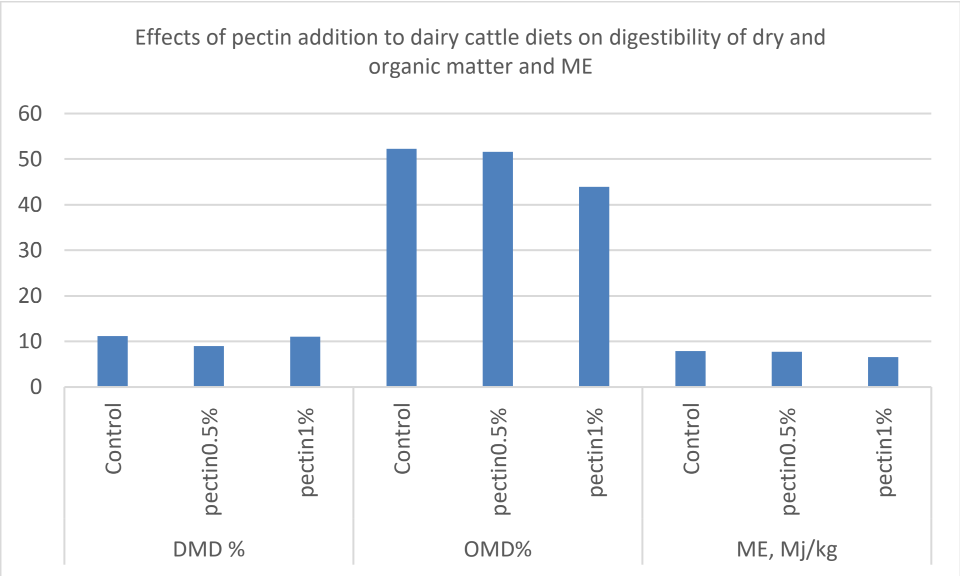
3.2.3. Effects of addition of pectin to dairy cattle diets on dry and organic matter digestibility and energy values

Effects of pectin addition to dairy cattle diets on digestibility of dry and organic matter, Metabolisable and net energy lactation values are given at Table 3.6. Dry matter digestibility of control were higher than other product (P<0.01). ME, MJ, NEL and ME (Kcal/kg) there were no significant differences among the control, pectin 0.5%, and pectin 1% products.

Table 3.6. Effects of pectin addition to dairy cattle diets on digestibility of dry and organic matter, Metabolisable and net energy lactation values

Item	**DMD, %**	**OMD%**	**ME, MJ/kg DM**	**NEL, MJ/kg DM**	**ME(kcal/kg)**
Control	11.14±0.09[a]	52.27±3.47	7.87±.55	4.61±.40	1880.00±131.13
Pectin 0.5%	8.97±1.07[b]	51.59±3.76	7.74±.60	4.52±.43	1849.33±141.82
Pectin 1%	11.05±0.23[a]	43.94±3.05	6.53±.48	3.63±.35	1561.33±114.89
Average	11.90±0.87	49.27±2.18	7.38±.34	4.25±.25	1763.56±82.37
P	0.001**	0.243	0.238	0.236	0.239

a,b,c,d: Differences between the averages are significant in the same column with different letter, GP: gas production OMD: Organic matter digestibility, ME: Metabolic energy, NEL: Net energy lactation, MJ: Mega joule, P: probability, **: significant at P<0.01.



Graphic 3.7. Effects of pectin addition on chemical composition of diets

CHAPTER 4

DISCUSSION

4.1. Experiment-I

In the planning of this study, it was emphasized whether there would be a decrease in the nutrient content and gas production potential of sugar beet pulp and the products obtained from the pulp, which is one of the important feeds commonly used in cattle nutrition. In addition, the effects of adding pectin obtained from SBP to dairy cow rations on nutrient composition, gas production and energy values were investigated (Doran et al., 2000). Especially since drying is an expensive process, most of the sugar beet pulp is sold as fresh and can only be stored in silos. On the other hand, biotechnological applications are carried out to convert this large amount of SBP into more valuable products other than feed without drying (Joanna et al., 2018; Usmani et al., 2022). Puligundla and Mok (2021) reported that the release of hydrolysis products, such as glucose from cellulose, as well as galactose, xylose, mannose, arabinose, and hemicellulose from pectin, is an important step to assess of SBP.

Sugar beet is a very common fodder material for ruminant animals in Turkiye. Ever year huge amount of sugar beet pulp produce in sugar companies. In an experiment (Şahin et al., 1999) it is determined that sugar beet pulp DM 10.79%, crude ash 8.54%, crude protein 8,62%, crude fat 1,27 % and crude cellulose content 24,95%. In another experiment (Yörük et al., 2014), it is found that sugar beet pulp's DM 14.89%, crude ash 0.41%, crude protein 1.27%, crude fat 0.36 % and crude cellulose content 2.74% in wet SBP. In the present experiment, SBP analyses showed that DM 89.78%, crude ash 4.59%, crude protein 8.63%, crude fat 0.56 % and cellulose ADF 24.84%. Protein concentration was very similar but crude fat and ash was different due to a possibility different cultivar of sugar beet and new technological processing technics. For example, ash content is closely related to mineral content of feeds and it may increase due to contamination of soil and other materials.

(Bosch et al., 2008)it is determined that sugar beet pulp fiber DM 92.0%, crude ash 0%, crude protein 9.6%, crude fat 0.4 %, OM 94.2 NDF 33.1 and ADF content 24.5%. In the present experiment, SBP diet fiber determined that DM 88.35%, crude ash 4.24%, crude protein 7.65%, crude fat 0.68 %, OM 84.12%, NDF 33.87 and ADF content 33.53%.

Dinand et al. (1999) reported that SBP consists of mixtures of polysaccharides about 1/3 cellulose 1/3 pectic polysaccharides (commonly called pectin). Bosch et al. (2008) determined that sugar beet pulp pectin’s DM 88.3%, crude ash 0.0%, crude protein 6.4%, crude fat 0.1%, OM 96.6% NDF 0.0% and ADF content 24.5%. In the present experiment, SBP pectin analyze showed that DM 88.26%, crude ash 7.97%, crude protein 5.75%, crude fat 0.43 %, OM 78.29%, NDF 0.0 and ADF content 0.0%.

Additionally, we have another product for experimentation with SBP cellulose DM 88.99%, crude ash 4.59%, crude protein 7.60%, crude fat 0.47%, OM 80.45% NDF 90.20% and ADF content 80.45%. Also we did SBP carbohydroxymethyl DM 87.39%, crude ash 4.24%, crude protein 0%, crude fat 0.23%, OM 83.15%, NDF 6.18% and ADF content 8.01%.

We are not aware to find an experimental results about SBP cellulose evaluation. Therefore we couldn’t compare our results with others.

In the current experiment it is determined that SBP gas production value for 6, 12 and 24 h, 11.50 ml,, 19.00 ml, and 49.00 ml, respectively. Kilic and Saricicek (2010) noted that SBP’s gas production values at 6, 12 and 24 hours incubation 16.82 ml, 29.73ml and 47.05 ml, respectively. While in another experiment (Getachew et al., 2004) reported SBP it is found that 6h contain 139.7 ml and 24h contain 286 ml. Bento et al. (2005) reported that gas production of cellulose contain 4h 1.77ml, 12h 2.21ml, 24h 45.5ml, while our experiment cellulose fond in 6h contains 7 ml,, and 12h contain 11.67 ml, and 24h contain 13.33 ml,. Additionally, we have another experimentation with Also we have CMC found 6h contain 2.50 ml, 12h contain 5.22 ml, and 24h contain 9.67 ml. Diet fiber fond 6h contain 8.33 ml, 12h contain 17.33 ml, and 24h contain 40.33 ml. Lastly pectin found in 6h contain 41 ml, 12h contain 62.67 ml, and 24h contain 70.67 ml.

Methane production in rumen composed of hydrogen (H) and carbon dioxide (CO_2).

Methane is produced by the reduction of CO_2 and H_2, which are formed as a result of anaerobic fermentation of nutrients (cellulose, hemicellulose, pectin and starch) in the rumen of ruminant animals, to CH_4 by methanogenic bacteria (Kebreab et al., 2006). The amount of methane gas produced by animals is affected by the type of carbohydrate in the diet, the amount of feed consumed by the animal, the productivity level, the speed of the feed passing through the digestive tract, the feed conversion rate, the saturation level of fat in the ration and the temperature (Navarro-Villa et al., 2011; Nkrumah et al., 2006). However, among these, reducing the H2 formed in the rumen or having a binding mechanism can reduce the methane gas formed in the rumen. Total methane emission from ruminants considered a significant source of anthropogenic sources of greenhouse gases. Moreover, the rumen-associated production of methane gases represents a loss of dietary gross energy for the animals, accounting for up to 12% of their energy intake. Also methane gas production of SBP of products (cellulose, CMC, diet fiber, and pectin). Hindrichsen et al. (2004) determined that methane production SBP contain 8.61ml while our experiment found 7.03ml. (Poulsen et al., 2012) found a methane production rate of 3.15ml in pectin. However, our experiment showed a higher methane production rate of 7.87ml in pectin. While our other excrement methane production fond cellulose contains 7.87ml and CMC contain 9.23ml and diet fiber contain 8.33ml.

Kilic and Saricicek (2010) found that *in-vitro* gas production techniques of different SBP showed that SBP of DMD contain 44.02%, OMD contain 62.35%, ME contain 9.26% NEL contain 5.46%. While our experiment SBP fond of DMD contain 9.85%, OMD contain 46.18% ME contain 6.86% NEL contain 3.88% and ME (kcal/kg) contain 1639.0%. Also we have other experiments like cellulose fond of DMD contain 2.51%, OMD contain 29.94%, ME contain 4.26% NEL contain 1.98% and ME (kcal/kg) contain 1018.3%. CMC fond of DMD contain 6.85%, OMD contain 16.74%, ME contain 2.24% NEL contain 0.50% and ME (kcal/kg) contain 534%. Diet fiber fond of DMD contain 12.83%, OMD contain 54.21%, ME contain 8.12% NEL contain 4.81% and ME (kcal/kg) contain 1942.3%. Pectin fond of DMD contain 16.45%, OMD contain 41.16%, ME contain 8.81% NEL contain 5.31% and ME (kcal/kg) contain 2105.7%.

4.2. Experiment - 2

The crude protein, crude fat, crude ash, ADF, NDF and OM analyzes of control and pectin with diet products in dry matter.

Bosch et al. (2008) it is determined that sugar beet pulp pectin 0% DM 88.3%, crude ash 0%, crude protein 6.4%, crude fat 0.1%, OM 96.6% NDF 0.0% and ADF content 24.5%. In the current study, an analysis of the diet composition of cows of control showed the following percentages: dry matter (DM) at 93.53%, crude ash at 6.72%, crude fat at 4.24%, organic matter (OM) at 86.80%, neutral detergent fiber (NDF) at 46.86%, and acid detergent fiber (ADF) content at 51.89%. The current study included the analysis of cow diets supplemented with 0.5% pectin. The results indicated that the dry matter (DM) content was 93.11%, crude ash content was 7.27%, crude fat content was 3.58%, organic matter (OM) content was 85.85%, neutral detergent fiber (NDF) content was 41.21%, and acid detergent fiber (ADF) content was 44.84%. In the current study, the analysis of the cow's diet supplemented with 1% pectin showed the following results: dry matter (DM) content was 90.91%, crude ash content was 8.09%, crude fat content was 3.80%, organic matter (OM) content was 82.82%, neutral detergent fiber (NDF) content was 48.12%, and acid detergent fiber (ADF) content was 50.39%.also determined pectin added dairy cow diets in crude protein were not found any significance.

In the current study, the addition of pectin to the diet affected gas production only in the first 6 hours with the addition of 0.1% pectin, but had no effect on gas production and methane production produced in 12 and 24 hours. This situation can be explained by the increase in the gas ratio in the presence of pectin, which is an easily soluble energy source in the first layer. However, it is important that methane did not increase in the total gas.Sunvold et al. (1995) reported citrus pectin con 6h 19.2 ml 12h 32.3 ml and 24h 52.8ml.while our control diet contain in 6h 14.67 ml also pectin 0.5% contain 6h 13ml another one pectin 1% 6h contain 23 ml while 12h and 24h gas production and methane production were not found any significant. We are not aware to find experimental results about pectin addition with dairy cattle diets on methane evaluation. Therefore we couldn’t find any paper result to compare the current results with others.

With pectin addition to dairy cattle diets on *in-vitro* digestibility of dry and organic matter digestibility, metabolisable and net energy lactation did not significantly changed. Since total gas and main nutrients such as protein, fat and ash were used in the calculation of energy values and the size of the differences between them was not significant, it did not significantly affect the results. Since there were not enough studies on the subject, a detailed discussion could not be added here.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

Research shows, sugar beet pulp and products with pectin added cow diet derivatives in cow diets affect rumen fermentation kinetics and in-vitro digestibility. Feeding studies and economic analysis are needed to prove sugar beet pulp is a feasible and cost-effective feed component. This research shows the potential advantages of adding sugar beet pulp to cow diets and emphasizes the necessity for complete and holistic analyses before commercial adoption.

Sugar beet pulp products increase digestibility, providing nutrient-rich, easily accessible nutrients for cattle. Fiber content supports microbial growth and nutrient utilization. However, long-term effects on cattle metabolism, digestive disorders, and nutrient imbalances must be considered. The impact of sugar beet pulp products on cattle diets, focusing on rumen fermentation kinetics and in-vitro digestibility. It revealed that sugar beet pulp products influenced microbial activity and fermentation patterns, leading to increased gas production and volatile fatty acid concentrations. This insight is crucial for optimizing feed formulations and supporting cattle health and productivity. The in-vitro digestibility experiments illuminated the nutritional value of sugar beet pulp products as a feed source. The increased digestibility observed can be attributed to the fiber-rich content of the pulp, which facilitates microbial growth and nutrient breakdown. This finding holds significant promise for enhancing feed efficiency and nutrient availability in cattle diets. This study shows promising results in incorporating sugar beet pulp products into cattle diets, but caution must be exercised in its practical implications. Long-term effects on animal performance, gastrointestinal health, and nutrient balance require further exploration. Feeding trials involving actual livestock are crucial to validate in-vitro effects and assess unforeseen consequences. Economic feasibility of integrating sugar beet pulp into cattle diets requires evaluation of factors like sourcing, processing, and cost-effectiveness. The study provides valuable insights into the dynamic interplay between sugar beet pulp products and cattle digestion, revealing their potential to improve rumen fermentation kinetics and in-vitro digestibility. This foundation for further research and informed decisions on incorporating sugar beet pulp products in cattle diets is essential for realizing the full potential of this approach for improving livestock nutrition and production.

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