Rumen metabolism and digestibility in dairy cows offered diets with forest by-products.

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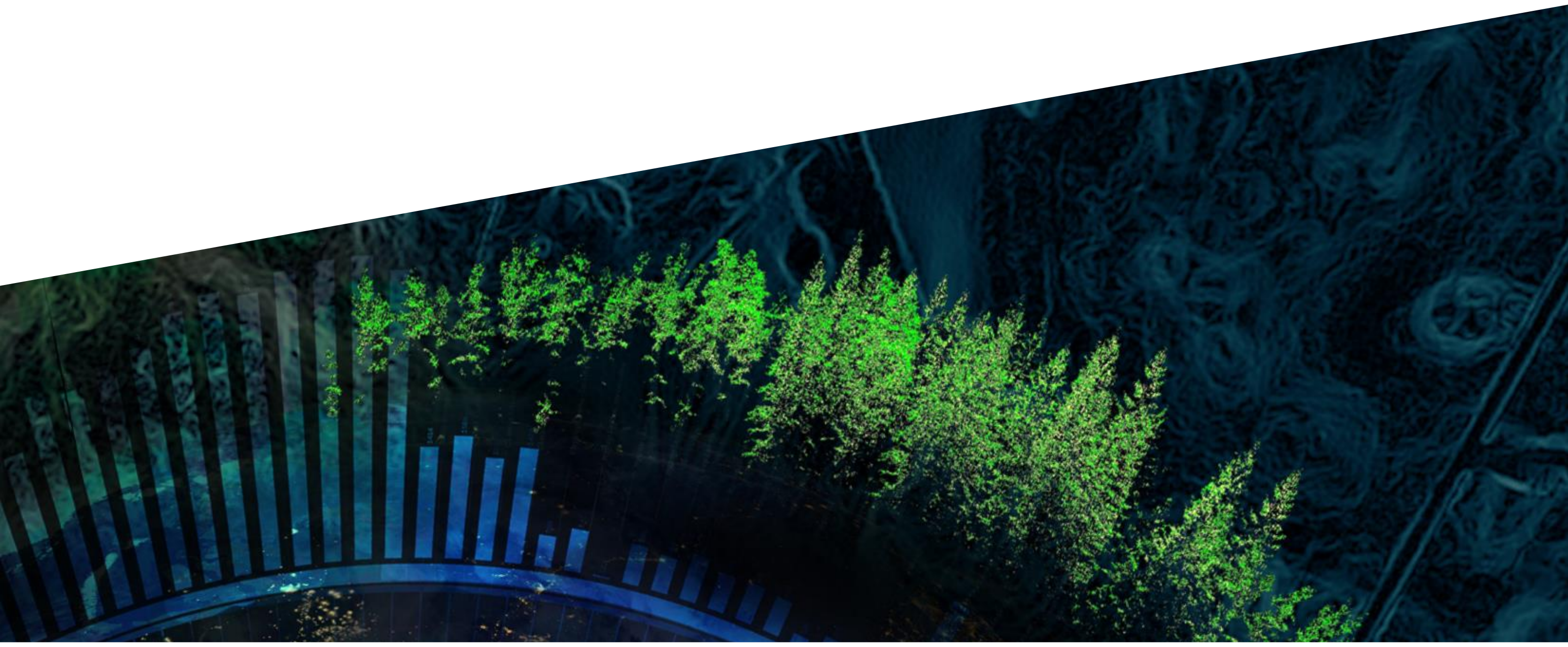

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Abstract

Swedish dairy industry faced a crisis of feed shortage in summer of 2018, followed by a dry autumn. The forage shortage was severe in some regions. During that period Swedish government intervened by introducing a 1.2 billion SEK package to help farmers and since then research is being done to find alternative feed resources in case of climate change effects in the future.

Sweden has a big matchstick industry that uses mainly aspen trees to produce matchsticks with the huge leftover aspen by-products comprising of bark and wood. In this context, the present study has been conducted to determine the potential use of aspen tree by-products as an alternative feed resource for lactating dairy cows. For this purpose, three lactating ruminally cannulated cows of similar age and days in milk were selected at Lövsta Research Station, Swedish University of Agriculture Sciences, Uppsala, Sweden. The selected cows were used according to a Latin square design with three diets, and three periods of three weeks each, comprising two weeks for adaptation and the last week for sampling. All three diets contained 9.70 kg/day DM of concentrate, plus: 1) Control diet, with approximately 12 kg DM/day grass silage; 2) Wood diet, with 6.7 kg DM /day grass silage and 4.1 kg DM/day ground aspen wood chips; and 3) Bark diet, with 6.7 kg DM/day grass silage and 4.6 kg DM/day ground aspen bark. During the sampling period (7 days) feed intake was calculated based on offered and refused feed quantities. Rumen evacuations, once per cow/treatment/period, were performed for the determination of the pools of dry matter, organic matter, and neutral detergent fiber. In addition, the ruminal liquid was sampled for pH. Fecal samples were collected to determine the digestibility of feed by acid insoluble ash.

Total dry matter intake was 21.86 kg/day for the control diet ($p \geq 0.08$) as compared to wood and bark with 19.23 kg/day and 19.76 kg/day, respectively. The crude protein intake was significantly higher ($p<0.02$) for the control diet with 4.17 kg/day compared to 3.16 kg/day for the wood diet and 3.26 kg/day for the bark diet. Total NDF intake was not different ($p \geq 0.14$) among treatments, with 9.42, 9.23, and 8.77 kg/day, for control, wood, and bark diets, respectively. Dry matter digestibility did not diff ($p \geq 0.31$), being 67 % for the control diet and 65% for both wood and bark diets. Milk yield was numerically higher ($p \geq 0.13$) for the control diet (29.42 kg/day) than for the wood diet (24.92 kg/day) and bark diet (26.23 kg/day). Rumen content (kg on fresh weight, FW) was similar for control and wood diets with 84.60 kg, while it was numerically lower ($p \geq 0.24$) in the bark diet (77.80 kg). The results for the ruminal content of DM and NDF tended to differ ($p \leq$

0.10), wood being higher, mean values 13.92 kg and 8.08 kg, than bark 12.43 kg and 7.13 kg and to control diet with mean 12.11 kg and 7.11 kg respectively.

Results of the present study suggest that the use of aspen by-products may represent an alternative source of roughage at times of fodder shortage. It is highly recommended, however, to check the level of crude protein in the diets as it is usually low in aspen by-products.

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1. Introduction

The World population has been growing dramatically and it is expected to increase from 7.6 billion in 2017 to 10 billion in 2067 (United Nations, 2017). For that, the dependability is now on the agriculture and livestock sector to ensure sustainable food production and alleviate the perils of hunger. Keeping in view the above population trend and rise in food demand, scientists and researchers have started looking for more sustainable and alternative feed sources for cows as livestock consumes around one-third of total cereal grains produced in the world. In developed countries, 70% of grains that are produced, are fed to animals among which 40% are fed to cows (FAO, 2002) which could have been eaten by humans.

The cattle industry is one of the biggest agricultural enterprises that contribute to dairy and other commercially important products for humans. There were more than 1.44 billion cattle animals across the world in 2014 according to United Nations. Asia alone accounts for 35% of these animals followed by 23% in South America, 12% in North America, 17% in Africa, and 10% in Europe. The demand for global milk production is estimated to increase by 48% in 2050 (Alexandratos and Bruinsma, 2012).

Livestock production can be improved when we can utilize available feed resources more efficiently. Ruminants' feed requirement is different at different stages of life. Feeds ingredients have different nutritional digestibility, and it is always a tough job to know which combinations of feedstuff are good for optimum livestock production. Roughages, plant-based foodstuff such as clover-grass leys, maize silage, and by-products of the industry as sugar beet pulp, as common examples, is natural sources of carbohydrates in animal's diets and affect their rumen activity either directly or indirectly. In dairy cows, roughages are responsible for sustaining the level of fat in milk, by helping to keep the rumen environment, stimulating rumination and salivation, and by producing acetate and other fermentation products in their rumen (Mendes Costa et al. 2020).

In case of roughages shortage, forest by-products are potential substitutes for roughages as a source of structural carbohydrates, although with poor digestibility. Forest by-products include wood residues, bark, logs, sawdust, wood chips, and leaves that are produced during harvest and postharvest of tree plants. Ruminants, due to the microbiome they host in their gut, can digest those to different degrees and produce milk and meat for humans (Takiya et al. 2019). Potentially, forest by-products could be utilized as an alternative source of roughage in ruminant diets (Röös et al. 2016).

During the summer of 2018, Sweden faced a forages shortage because of a combination of low rainfall with high temperatures. This made Swedish farmers, advisors, and researchers think about alternative ways of feeding cattle. Trees, available in abundance, can be used as an alternative feed source for cows.

The major portion of forest by-products i.e., trees are composed of structural carbohydrates such as hemicellulose and cellulose and lignin. There is a strong chemical bond between the carbohydrates and lignin complex which makes its digestibility limited (Fox & McDonald, 2010). Aspens are tree species that belong to the *Populus* genus that have a prime role in maintaining biodiversity. Aspens exhibit many ecological services that include carbon sequestration, re-vegetation, and production of novel wood material (MacKenzie, 2010; Boča and Van Miegroet, 2017). Aspen is native to North America and some are also found in South America, Asia, Japan, North Africa, Sweden, and other European countries (Rogers et al. 2020). In North America, it occupies the portion of the Midwest, the Black Hills, the Rocky Mountain, and the Great Lakes region (Dudley et al. 2020).

In Sweden, the growing stock of aspen trees represents 1.8% of the total forest trees in the whole country. In the Svealand, aspen tree contributes 2.8 % of that area of forest trees, followed by Gotland 2.3%, then South Norrland contains 1 %, and the least is found in Northern Norrland 0.8 % (official forest statistics Sweden 2021). It is a fast-growing and less lignified tree. Enzmann et al. (1969) checked the chemical composition of aspen bark and found out it contains cellulose (36%), hemicellulose (13.9%), and lignin (23.1%).

Aspen wood chips and aspen bark are by-products from the matchstick industry that may have potential as an alternative roughage for cattle diets in shortage situations. The objective of the project was to study the effect of the inclusion of aspen by-products into a grass-silage-based diet fed to lactating dairy on intake, diet digestibility, rumen metabolism and milk yield.

The general aim of this project was to investigate how aspen by-products will affect the performance of dairy cows? The hypothesis was that aspen by-products can be used as a partial roughage substitute (up to 6 kg DM grass silage per day replaced by aspen by-product) in diets for dairy cows.

2. Review of Literature

2.1 Feed particle size and intake

Roughages are plant-based products with high fiber content, quantitatively cellulose and hemicellulose, as forages, grasses, legumes, and cereal kernel crops if harvested as a whole crop. Roughages are important dietary components in ruminant feedstuffs and are necessary to support normal rumen physiology and function to maintain health (Mirzaei-Aghsaghali & Maheri-Sis, 2011). They have physical as well as chemical features that are necessary for digestion (chewing during rumination) and enzymatic degradation during rumen fermentation. The fiber in roughages is commonly measured as neutral detergent fiber (NDF) and acid detergent fiber (ADF). NDF contains lignin, hemicelluloses, and cellulose portion of feed and they are comparatively more related to chewing activity than ADF (Mertens, 1997). ADF contains cellulose as a primary constituent and lignin that affects digestibility (Van Soest, 1965; Mertens, 1997). The structure of the feed has a great impact on rumination, therefore affecting certain parameters such as feed intake, chewing activity, rumen pH, animal milk fat, and its profile of fatty acids. Kononoff et al. (2003b) reported that fiber particles size of corn silage in lactating dairy cows (geometric means from 7.4 to 8.8 mm) does not have an effect on the rumen pH but found quadratic effect on the milk fat level but no effect on the milk protein level. In another study was done by Kononoff et al. (2003) feeding alfalfa silage to the cows in early lactation reported that reducing particle size does not affect pH, milk yield, and milk fat but it did affect the level of protein. On the other hand, Grant et al. (1990) reported that smaller size forage particles have a negative effect on milk fat, as smaller size particles move out from the rumen quickly, reducing rumination and saliva production, important to maintain the rumen pH.

The fiber content and particle size are important factors affecting feed intake and thus the diet digestion in dairy cows (Tafaj et al. 2005). A smaller particle of feed will move out more quickly from rumen which results in less chewing activity, less saliva secretion, and reduced ruminal contractions for mixing the feed with saliva and water (Mertens, 1997). Feed type, its intake, and the physiological condition of the animals are key factors that influence chewing activity. Chewing activity determines the quantity of saliva production. Feed that promotes chewing activity is found to be necessary for rumen function as saliva acts as a buffer for the rumen liquor, keeping rumen pH within a normal physiological range, and milk fat secretion in lactating dairy cows (NRC, 2001; Moran, 2015). The intake of feed by the cows depends upon the size and frequency of particles size of fibers in the diet. To understand the effect of particle size on the feed intake, Kononoff et al. (2003b) experimented with three different particle size of corn silage i.e., 1.18,8 mm, and 19 mm. The author found out that dry matter intake and chewing activity for the cows were increased for the size 1.18 to 8 mm but when the size of particles was increased more than 19 mm in the diet, the intake of feed was reduced as it can be due to gut fil condition with more longer particles in the diet. High producing dairy cows will require larger and more frequent feed intake than low-producing cows. To avoid rumen acidosis in high-producing lactating cows, a particle size ranging from 1.18 mm to 8 mm would be beneficial, as it enhances DM intake and production. Another study done by Zebeli et al. (2010) reported that particle size bigger than 8 mm may reduce DM intake and production.

2.2 Physical structure of feed in the rumen

As a result of feed degradation and rumen stratification feed being digested in rumen appears in different forms such as packed mat, liquid, or gas. The stratification depends on the dry matter (DM) contents of the feed and these contents are higher in the medial rumen. Further to this, stratification may also depend on the size and density of particles bigger and less dense particles will be on the top, while smaller and more dense particles will be at the bottom. Rumen mixes the ingested food and the rumen content which results in a ruminal mat (thick mass of digesta) in the medial rumen as it has more DM content. Medial rumen has a denser packed mat than ventral rumen which has most of the liquid (Evans et al. 1973; Tafaj et al. 2004; Hummel et al. 2009). The feed particles can either float or sediment depending on their density and buoyancy (Jung and Allen, 1995). After fermentation of the mat large amounts of gas are released but some of the gas becomes trapped on the mat particles, making them buoyant. The particles from the mat gradually lose their buoyancy and get into the ventral rumen by ruminal contractions. Then after rumination, the particles get back into the dorsal rumen where they get mixed with liquid. The upper part of the rumen is filled with gases that are frequently expelled through the mouth (Schulze et al. 2014).

2.3 Microbes in rumen

A variety of microorganisms are found in the rumen of animals which is crucial for their ability of feed utilization, digestibility, and health (Wang et al. 2020). These microorganisms include bacteria, protozoa, archaea, fungi, and viruses. Bacteria and protozoa account for the maximum number (80%) of these microbes in the rumen and have the ability to digest fibers (Sha et al. 2020). These microbes have particular enzymes that perform amylolytic, fibrolytic, and proteolytic activities to digest non-structural and structural carbohydrates and proteins. *Fibrobacter succinogenes, Clostridium longisporum, Ruminococcus albus, and Butyrivibrio fibrisolvens* are important cellulolytic bacteria that digest cellulose in the rumen (Dehority, 1993; Miron and Ben-Ghedalia, 1993). These bacteria play an important role in digesting and fermenting polysaccharides and protein to generate energy for themselves and the host animal (Deusch et al. 2017). Moran (2005) reported that 70 to 80% of digestible DM is digested in the rumen by bacteria and protozoa.

Protozoa are also involved in cellulose, starch, and protein digestion through phagocytosis (Chesson and Forsberg, 1997; Williams and Coleman, 2012). *Dasytricha and Isotricha* are predominant protozoans for carbohydrate fermentation and protein digestion. Archaea microbes, also termed methanogens, are important for producing gasses such as methane. These microbes are attached to ciliates protozoa and synthesize methane. According to Hegarty (1999), methanogens present in the rumen and also living on the surface of rumen ciliate protozoa are responsible for rumen methane emissions and he concluded that removal of protozoa has benefit in reducing the greenhouse gases and is beneficial for livestock production as it provides more protein to the host animal which otherwise would have been used by protozoa. These microbes are important for the proper functioning of the rumen (Wright, 2015).

Anaerobic fungi hold a very small portion of microbiota and have lignin-cellulose degrading enzymes that digest polysaccharides i.e. plant fibers along with bacteria, archaea, and protozoa (Gruninger et al. 2014; Puniya et al. 2015). All these microbes perform synergistically in the proper functioning of the gastrointestinal tract of animals (Kumar et al.2015).

2.4 pH of rumen

The rumen pH ranges from 5.7 to 7.3 varying during the feeding cycle of animals (Hespell et al. 1997). Rumen and reticulum microbiome work properly in the ranges of 6 to 7 (Moran 2005) and McDonald et al. (2010) reported that normal rumen pH remains between 5.5 to 6.5. The temperature of rumen remains between 38 to 40° C (McDonald 2010; Moran 2015). Rumen pH is important for the proper functioning of microorganisms. Optimum pH differs among different microbes and but most can´t survive in a highly acidic or basic environment. Rumen pH is affected by various factors that include diet, feed intake, the fiber content of the diet and particle size, rumen degradation rate, and end products and their absorption (Banakar et al.2018). When cows are fed diets rich in non-structural carbohydrates, i.e., sugar and starch, the rumen pH decreased for a few hours because of the production of end products. Then it increases again following the removal of fermentation end products. High-producing lactating cows tend to have rumen acidic pH because of the high food intake of diets with relatively low fiber content that disturbs buffering system of the rumen (Palmonari et al. 2010). This can cause acidosis (pH <5.6) condition that disturbs the activity of microorganisms (Aikman et al. 2011). Volatile fatty acids lower the rumen pH, which has a large effect on microbial protein (MP) synthesis and its efficiency (Piwonka et al. 1994). Low pH negatively affects many of the major ruminal cellulolytic bacteria species; cell yield of *Ruminococcus flavefaciens* decreases (Shi and Weimer, 1992), glucose transport of *Fibrobacter succinogenes* is inhibited (Chow and Russell, 1992), and fiber digestion decreases (Shi and Weimer, 1992). As different microbes work at different pH values, changes in pH can change the relative proportions of different microbe species in the rumen consortia. Absorption of the end products of microbial fermentation from the rumen is important as accumulation of VFA in the rumen can lower the pH resulting in a disturbance of the rumen microbiota (low pH less favorable for fibrolytic activity of bacteria) and hence digestion of feed can be affected (Dijkstra et al. 2012). Protozoa digest starch more slowly than bacteria and the presence of a high amount of protozoa may result in a reduction in the availability of MP because protozoa have a locking effect on the microbial protein, and it reduces the passage of microbial protein to the small intestine. Defaunation (removal of protozoa from rumen) has resulted in 25% more microbial protein reaching the intestine (McDonald 2010) and hence animals can utilize that microbial protein, and its productivity can be increased (Hegarty, 1999).

2.5. End products of roughage digestion

The end products of roughages digestion, volatile fatty acids, are predominant sources of energy for the host animal. The rumen microbes digest carbohydrates and other feed content in a process of fermentation and produce three main volatile fatty acids (VFAs) i.e., acetate, propionate, and butyrate. These VFA's are a major source of energy for the host which are absorbed through ruminal wall (McDonald 2010) and contribute almost 70% of digestible energy (Moran 2005). A balanced forage-based diet is needed for normal rumen function, as diets containing more starch or sugar and less fibrous diet can cause acidic pH which is unfavorable for cellulolytic bacteria present in the rumen (Moran 2015). Other than VFA's, cattle also produce methane and CO2. The ratio of these gases is 40% carbon dioxide and 30 to 40% methane, about every 100g of carbohydrate digestion produces 4.5 g of methane and that represents loses of around 7 to 13 % of gross energy of feed in form of methane (McDonald, 2010). These gases are found above the liquid and solid contents of the rumen. VFA and gas production is correlated (Williams et al. 2019).

2.6 Rumen retention time

Forage particle size influences the digesta´s residence time in the rumen. The normal retention time in the rumen is 30 to 80 hours and the digesta particulate matter passage rate is 0.012 to 0.03/hour (McDonald 2010). In a study on the effect of particle size of fibers on the rumen retention time, it was concluded that the retention time in rumen increased from 31.7 h to 38.4 hours with long particles (19 mm) compared with shorter particles (4 mm) (Ramírez Ramírez et al. 2016). Poppi et al. (1981) found the rumen retention time for longer particles to be between 16.1 to 20.2 hours. However, long particles alone are not responsible for rumen retention time but also the small particles <1.8 mm which make up almost 72% of the dry matter content in the rumen. Jiang et al. (2018) also found a similar relationship between particle size and rumen retention time. The same author suggested that smaller particle size will increase the ruminal passage rate which in turn can affect digestibility and feed intake.

2.7 Function of the rumen, reticulum, omasum, abomasum, and intestine in digestibility and nutrient absorption

Feed stays in the rumen for a varying time depending upon the particle's size. The rumen retention time was between 16.1 to 38.4 hours for the small size and large size particles, respectively, i.e. <1.8 mm to 19mm (Ramirez Ramirez et al. 2016; Poppi et al. 1981). So, after some time in the rumen feed digesta moves into the next compartments. The rumen and reticulum are considered as a single organ for the mixing of consumed feed and the microbial digestion. According to Moran (2005) between 70 to 80% of digestible dry matter is digested in the rumen. Digesta after mixing by rumen contractions passes into the omasum via the reticulo-omasal orifice. Omasum contains leaves that are involved in the absorption of water, VFAs, inorganic electrolytes, ammonia, and further filtration of digesta. Then digesta passes into the next compartment, the abomasum that works exactly like a glandular stomach that is also termed as a true stomach. It releases its own enzymes and acids and digesta is further mixed through muscular contractions before entering into the intestine. The small intestine consists of three sections, first digesta pass into the duodenum and then the jejunum and finally the ileum by peristaltic contractions. Then digesta move into the large intestine where occurs some microbial fermentation, mixing of the digesta is further done through peristaltic as well as antiperistaltic contractions mix it well and further water and VFAs are absorbed from it (Dijkstra et al. 2005).

3. Wood and bark as ruminant feed

3.1 Chemical composition of aspen by-products

The fiber fraction of feed for ruminants is normally analyzed and expressed as neutral detergent fiber (NDF) and acid detergent fiber (ADF). The NDF main components are cellulose and hemicellulose which are potentially digestible and indigestible lignin. The ADF comprises cellulose and lignin portion of the feedstuff. Yemele et al. (2008) reported the chemical composition of aspen bark, on a DM basis, as cellulose (25.4%), holocellulose (hemicellulose and some part of cellulose) (48.8%), lignin (22.6%) and together with some other components (other than cellulose, hemicellulose, and lignin). Heroy et al. (2016) reported that, on a DM basis, aspen bark contains hemicellulose 11.3% and cellulose + lignin 52.6%. Claude et al. (2008) showed that trembling aspen bark comprises 66.2% of holocellulose (hemicellulose and some part of cellulose), 34.5% of cellulose, and 30.7% of lignin-based on extractive-free bark. Kipp (1979) studied aspen wood and found on a DM basis, 15.8% hemicellulose, cellulose 46.5 %, and lignin 16.9 %. Enzmann et al (1969) reported that aspen bark contains cellulose (36%), hemicellulose (13.9%), and lignin (23.1%). Hence, aspen sawdust (wood shavings) can also be used as a roughage substitute because it contains structural carbohydrates such as cellulose, hemicelluloses, lignin, and a minor amount of extractives (Horisawa et al. 1999).

3.2 Aspen by-product digestibility

Forest products have generally low digestibility. There are mainly two types of wood i.e., hardwood and softwood. On the contrary, as the name suggests hardwood is relatively more digestible than softwood because the prior contains more cellulose and less lignin. Aspen tree is one of the hard types of wood suitable for use as a feed, due to the high *in vitro* digestibility of the dry matter, which is 33 % for the wood and 50 % for the bark (Baker et al. 1975).

The *in vivo* digestibility in goats of aspen wood DM was slightly higher, 41% as reported by Mellenberger et al. (1971) (Table 1). Wood digestibility is usually low. Millet et al. (1970)

assessed the digestibility of 24 species of trees and found that very few were digestible in the cows' rumen i.e., aspen and maple. However, he also mentioned that if the less digestible species of the trees are treated with different chemical or physical methods, their digestibility can be increased. Mellenberger et al. (1971) pointed out that sodium hydroxide treatment of aspen sawdust, enhanced the digestibility of dry matter, energy, and carbohydrate in goats. Both authors reported an increase in the NDF and ADF digestibility when aspen sawdust was treated with chemicals. Muyng et al. (1988) compared the effect of 20 % alkaline hydrogen peroxide (AHP) treated aspen feed with untreated aspen feed in an experiment with sheep. Feeding of AHP treated aspen ration had more organic matter digestibility, NDF digestibility, and feed gain ratio than untreated aspen sawdust. With his results, he concluded that AHP treated aspen sawdust is more beneficial for ruminants, and in experiment two in which he used a nylon bag to check the effect of AHP treated aspen on non-lactating cows, concluded that AHP has a role in breaking the lignocellulose bond and ruminants can better utilize AHP treated aspen sawdust. The same researcher reported that when the rumen retention time increased, the digestion of DM and CP was reduced in the intestine, as more digestion happened in the rumen.

Satter et al. (1970) prepared a ration with high grains for lactating cows and partially replaced roughage with aspen sawdust in three groups of rations with lactating dairy cows. From three experimental diets, the one with 32 % aspen substituted ration (DM basis), significantly increased milk fat % but no effect on the milk production and digestibility of that aspen substituted ration was relatively less as compared to the other two rations and to compensate the lower digestibility animals have eaten more aspen substituted ration and the authors draw the conclusion aspen sawdust can constitute 30% of the ration for lactating cows without any negative effects on the milk production (Satter et al. 1970).

In lactating cows, Baker et al. (1975) reported that dry matter digestibility of aspen sawdust was 35 % and of aspen bark 50 %. Goodrich et al. (1977) reported that aspen bark silage can be used in beef cattle as an alternative feed resource and he advised feeding the aspen bark silage in grounded form for better intake and digestion. Fisher (1980) experimented with lactating cows with a diet of 23 % aspen (steam processed), 74% corn silage, and 2.3 % soyabean (protein source) as feed ingredients. The composition of the steam processed aspen was 45.4% DM, protein level was 0.54% DM and ADF 73.7% DM. Results indicated that DM intake and milk production was higher in the aspen substituted diet as compared to the control diet. Kipp (1979) studied if aspen wood pellets could be used as a partial roughage feed source in lactating dairy cows. The experimental units were divided into two groups, group 1 cows were fed diets with 30% DM from aspen pellets, 30% DM from corn silage, and 40% DM from 28% CP mix concentrate, and group 2 was fed with 40% DM from mix concentrate and 60% DM from the corn silage. Aspen contained 16.9% lignin, 1.9% crude protein, 80.3% NDF, and 64.5% ADF. Results revealed that there was no considerable effect on milk yield, milk composition, and milk flavor but there was a slight rise in fatty acid concentration. The author concluded that aspen pellets can be used as a partial roughages source for lactating dairy cows (Kipp, 1979).

Table.1 The dry matter digestibility % of aspen bark or wood

Parameter	**Wood**	**Bark**	**Technique**	**1%NAOH treatment**	**References**
Dry matter-digestibility %		31	*In vivo* sheep	N/a	Enzmann et al 1969
	33	30	*In vitro*	>50	Millet et al 1970
	41	50	*In vivo* goats	>50	Mellenberger et al 1971
	35	50	*In vitro* & *In vivo* (cows)	>50	Baker et al 1975

Table 1 shows values of DM digestibility of aspen tree i.e., wood or bark reported in the literature. Mellenberger et al. (1971) reported that the digestibility of aspen can be improved by a factor of 25 % if treated with alkali i.e., 1% NaOH. Another study by Fullinwider (1976) on goats showed that the DM digestibility with aspen bark was 50 %. Forsberg (1977) experimented with steers fed processed aspen wood diet (with 15% to 30% of DM, by replacing corn silage roughages) on the feed intake and feed/gain ratio as compared to the control diet (corn silage with combinations of different feed ingredients i.e., barley, soyabean). His trial showed that the processed aspen wood diet did not affect the feed intake, but the aspen substituted diet has significantly $p<0.05$ increased the feed gain ratio with a 30% aspen wood diet as compared to the control diet. The same author reported that aspen substituted diets had lowered the DM digestibility, but the digestibility of crude protein and crude fiber were not affected as compared to the control diet.

4. Materials and Methods

The experiment was carried out at the Swedish Livestock Research Centre, Lövsta, Swedish University of Agricultural Sciences, Uppsala, Sweden (59°50′N, 17°46′E). The Uppsala Ethics Committee approved the experimental protocol and all handling of animals (no.5.8.18-18643/2020 003488). Three lactating dairy cows tagged as 451, 605, and 743 of the Swedish Red breeds (804 ± 72 kg of BW; 156 ± 15 DIM; 34.5 ± 1.1 kg milk/d expressed as mean ± standard deviation at experimental onset) were used. All cows were fitted with rumen cannulas with 100-mm i.d. (Bar Diamond Inc., Parma, ID).

The study was conducted according to a 3x3x3 Latin square design (3 diets, 3 cows, 3 experimental periods). Each of the experimental periods lasted 3 weeks. The first two weeks were used for the adaption of the animals to the treatments. Data and sample collection were done during the third week. The experiment was preceded by a preparation period lasting from 2021-01-22 until 2021-02-12. During the preparation period, cows were transferred to the experimental area, trained to locate their individual feeding troughs, and introduced to minor amounts of the wooden materials to be fed in the experiment. The cows allocated to wood and bark diets were at the end of the preparation period allowed to 1.5 kg of the respective product. The experiment (9 weeks) started on 2021-02-13 and ended on 2021-04-17. During each period sampling was done like feed sampling, rumen sampling for RNA analysis (not reported in this thesis) and pH measurement, total rumen evacuation, and fecal sampling. There were 3 sampling periods: Period 1 started on 2021-02-26 and ended on 2021-03-05. Period two started on 2021-03-19 and ended on 2021-03-26 and period 03 started on 2021-04-09 and ended on 2021-04-16.

Table 2. Periods, cows ID, and treatments throughout the experiment.

Period	Cow	Treatment	Adaption Period	Sampling Week
1	451	Control	two weeks	2021-02-26 to 2021-03-05
1	605	Wood		
1	743	Bark		
2	451	Bark	two weeks	2021-03-19 to 2021-03-26
2	605	Control		
2	743	Wood		
3	451	Wood	two weeks	2021-04-09 to 2021-04-16
3	605	Bark		
3	743	Control		

Throughout the experiment, the cows were kept in a separate area of the loose housing barn, with access to a water bowl, a concentrate feeding station, and two forage feeding troughs per cow (CRFI; BioControl, Rakkestad, Norway). The bedding material was sawdust. The feeding troughs were automatically controlled, and we used cow transponders for feed allowance restriction and also to avoid cows from eating each other diets from different feeding troughs. The feeding troughs contain a screen that shows how much feed was fed and how much it is left in the trough, so feed intake was recorded automatically. The feed leftovers were recorded manually by the staff. The cows usually have access to the feeding area and resting area 24/7. The cows were manually moved to the adjacent AMR for milking at 0600 and 1800 h.

All diets contained 7 kg concentrate Komplett Xtra 205 (basal concentrate), and 3.5 kg concentrate Konkret Mega 28 (protein supplement) (both from Lantmännen, Stockholm). A different batch of Komplett Xtra 205 was fed in the milking robot and hence chemical composition is presented separately for the milking robot There were three diets as fed basis, Control diet: 40 kg grass silage, Wood diet: 22 kg grass silage with 8 kg aspen wood chips, Bark diet: 22 kg grass silage with 8 kg aspen bark.

Table.3 Feed allowance as fed basis (mean SD values, kg/day) for cows during the sampling periods.

Feed	**Control Mean**	**SD**	**Wood Mean**	**SD**	**Bark Mean**	**SD**
Silage	39.67	0.81	22.42	1.66	22.40	0.38
Wood	-	-	8	0	8	0
Bark	-	-	8	0	8	0
Basal concentrate	4.01	0.04	1.99	0.01	1.99	0.01
Protein supplement	3.55	0.07	3.49	0.03	3.50	0.02
Basal concentrate trough*	2.90	0.30	3.90	0.75	4.35	0.77

*Basal concentrate mixed with wood or bark.

Table 4. Chemical composition of the different components of the diets.

Parameters (%)	**Silage**	SD	**Basal concentrate**	SD	**Protein-supplement**	SD	**Basal concentrate trough**	SD	**Bark**	SD	**Wood**	SD
DM	30.73	0.26	86.86	0.26	86.84	0.71	87.28	0.46	51.64	0.39	57.2	0.98
CP	16.37	0.01	20.53	0.16	26.48	0.11	20.48	0.23	2	0.23	0.93	0.44
NDF	60.51	4.78	19.48	0.87	20.37	1.89	23.29	1.49	71.57	1.54	87.95	1.7
AIA	1.79	0.04	0.56	0.07	0.73	0.11	0.59	0.12	0.15	0.07	0.02	0.01
Ash	10.4	0.15	6.84	0.04	7.22	0.36	8.75	0.14	2.62	0.32	0.63	0.19

Neutral detergent fiber (NDF) values were highest in the wood compared to bark and control diet (Table 4).

Aspen by-products were from matchstick manufacturing (Swedish Match, Vetlanda). They were stored frozen. Before feeding, wood or bark samples of aspen trees were stored at -22 C, later on, thawed, and milled through an 8 mm screen on a hammermill. Consumption from forage troughs concentrate station, and allowance in milking parlor were recorded automatically. Forest by-products i.e., wood and bark samples were sampled at milling two times during each period i.e., at the start of the period and at the end of the period. Silage and concentrates were sampled from forage troughs and feeding stations respectively, every day during the sampling week from Monday to Friday. There was a recording of data on how much feed was fed and how much was left. Later on, the samples of refused feeds i.e., silage and bark /wood leftovers were taken. For the leftover's wood/bark sampling, we had buckets specifically labeled as per cow and period during the sampling week. The feed refusals, either silage, wood, or bark leftovers were mixed thoroughly before taking one representative sample. These were later stored in a deep freezer at -22 C at the Lövsta research station until analyses.

Rumen evacuation was done at the start of the period usually every Friday i.e., before the sampling week starts and at the end of sampling week. Before rumen evacuation, rumen liquid sampling was done to check the pH of the rumen. Rumen evacuation procedure was simple, taking the fresh rumen digesta out from the rumen and weighing it, and then putting back all the digesta in the cows. During rumen evacuation, every tenth handful of samples was put into a separate bucket for rumen contents to be sub-sampled and frozen for later analysis. For rumen liquid sampling, small containers (150 ml) were used to take out the rumen content from the ventral side and then processed by straining through a tea strainer, pH was measured at different intervals of time during the sampling week (Mettler Toledo MP 125, Mettler Toledo AG, Schwerzenbach, Switzerland) and poured into the tubes which were later stored at -22 C. For pH data, time was recorded when we took the rumen liquid sample from the cows, and this was done during the sampling period between 0500 to 2000 hrs. at 05 hours intervals. All the data was recorded manually as per the already scheduled timetable. Fecal samples were taken two times a day around 0600 to 0900 hrs. in the morning and 1500 to 1700 hrs. in the evening. Preference was given to take voluntarily defecated fecal samples but if the cow did not defecate within the time frame, then the sample was taken from the rectum. All the collected samples were stored in the freezer at -22 C.

The actual diets given to the cows when fully adapted after two weeks in each period were in kg of fresh weight/d. Rumen evacuation procedure was simple, opening the cannulated lid, taking the contents out, every tenth handful from the rumen was put in a separate bucket for sampling. During period 3 there was electricity failure, and it was relatively shorter comprising only four days. The normal rumen liquid sampling for pH measurement, noting of the fresh weight of the rumen digesta, and then feed refusals sampling data, all were initially recorded manually and then stored in an Excel sheet. It was counterchecked to avoid the record discrepancy.

4.1 Preparation of samples for laboratory

The feed samples, which were stored in a deep freezer -22 C, were prepared for analysis in the laboratory. There were five feed intake samples i.e., silage, wood, bark, basal concentrate, and protein supplement. Wood and bark samples were taken in each period two times, at the start of the sampling period time and the end of the period, then these two samples were thoroughly mixed, and out of two as per period, one representative sample was made and sent to a lab for analyses. Silage, basal concentrate, and protein supplements samples were taken each day during the sampling period from Monday to Friday, so a total of five samples from each period, which were thoroughly mixed, and one representative sample for each period were sent to the lab for analyses. Rumen liquid samples were taken three times a day after a 05-hour interval during the sampling period. Rumen liquid content after straining was poured into three Eppendorf tubes and one test tube in every sampling in each period. These samples were then sent to a lab for analysis. There were feed refusal samples from wood, bark, and silage. Silage, wood, and bark samples were collected from respective troughs every morning before 0900 hrs. during the sampling period as per cow specific which was later on mixed thoroughly for each period and made one representative sample for each period. The fecal samples which were poured into the plastic bags were pooled from Monday to Friday for each cow as per period and then it was mixed with 10 % water on a fresh weight basis. The pooled samples were then drilled with a manual hand driller until it was uniformly mixed. The duplicate Petri dishes samples were taken for each cow during each sampling period and then these were sent to a lab for analysis.

4.2 Analyses

Feed samples were first dried at 60°C in an oven for 24 h, it was grounded in a hammer mill (1-mm screen) and analyzed for DM by drying at a temperature of 105°C overnight. CP was measured by a fully automated Kjeldahl procedure (Technicon, Solna, Sweden). The NDF was measured by a method described by Murphy et al (2000) with α-amylase (Termamyl®, NOVO Nordisk A/S) for the feed samples. The acid-insoluble ash was measured by van Soest et al (1991) standard methods. Fecal samples were also analyzed for DM, NDF, OM, and CP to check the digestibility, as was done for the feed samples. Rumen contents were analyzed for DM, OM, NDF, and ash, which were planned to check rumen passage rate and rumen fill rate, but these are not presented in this thesis. Milk composition i.e., fat, protein, and lactose were determined by automated infrared analysis (Dairy Lab 2, Foss Electric, Hillerød, Denmark)

4.3 Calculations and statistical analyses

The feed intake and milk yield were recorded automatically in the troughs and voluntarily milking unit, respectively. The feed refusals were recorded manually. Energy Corrected Milk (ECM) was calculated according to Sjaunja et al. (1991) as:

$$\text{ECM} = \text{milk yield (kg)} \times \{[383 \times (\text{fat }\%) + 242\times (\text{protein }\%) + 165 \times (\text{lactose }\%) + 20.7]/3140\}.$$

Statistical analyses were performed with the computational SAS software (version 9.4 SAS Institute Inc., Cary, NC)), according to a Latin Square design, by Proc MIX with treatment and period as fixed classes and cow as random. Significance was declared at p-value < 0.05 and trends were reported with the p-value <0.10.

5. Results

5.1 Feed Intake

Due to the difference in chemical composition and feed allowance among diets, intake and CP differed, it can be because of allowance-dependent difference in silage intake (silage part on the fed basis was intentionally reduced to 22 kg/day for wood and bark diet each and 40 kg/day for control diet on fed basis) and the difference in crude protein intake caused by less crude protein in the wooden by-products than in the silage (Table 6). The concentrate DM intake with the wood by-product treatments was 0.6 – 1.0 kg less numerically than for the control diet (Table 6). These numerical differences were also reflected in the intakes of total dry matter, organic matter, and NDF.

Table 5. Feed intake (as fed basis, mean SD, kg/day) by cows during sampling week.

Diets	Silage		Basal concentrate		Protein-supplement		*Basal conc in the trough		Wood		bark	
	mean	SD	mean	SD	mean	SD	Mean	SD	Mean	SD	mean	SD
Control	39.67	0.82	4.01	0.03	3.55	0.07	2.9	0.3				
Wood	22.41	1.65	1.99	0.01	3.49	0.03	3.9	0.74	6.24	1.19		
Bark	22.4	0.39	1.99	0.01	3.5	0.03	4.36	0.76			6.97	1.23

*Extra allowance of basal concentrate mixed with either bark or wood in the trough.

Feed intake of different ingredients of diet is presented in the Table 5. The interesting point is, that cows were offered an amount of concentrate mixed in either bark or wood, to make cows eat. The bark diet mixed with concentrate has a more concentrate intake 4.36 kg/day as compared to wood with a mean of 3.9 kg/day. During period 2 cow 451 had eaten the maximum of the conc/bark portion of the diet on a fed basis of 13.04 kg/day while the minimum was found in period 3 where cow 743 had consumed the lowest wood/conc portion of the diet on a fed basis which is 7.98 kg/day.

5.2 Feed Digestibility

There were only numerical differences ($p \geq 0.20$) between the treatments regarding the digestibility of DM, OM, NDF and CP (Table 6). The largest range of values was for the NDF fraction, which spanned from 58% (bark) to 64% (control), while the other digestibility measurements were more similar.

5.3 Milk production and composition

The milk production and composition results were not significantly different among treatments ($p \geq 0.24$), with kg milk/d ranging from 29.42 for the control to 24.92 kg/day for the wood diet, and kg ECM /d from 29.61 for the control to 25.63 for the wood (Table 6).

5.4 Rumen

We measured and analysed different parameters such as DMI, rumen pH, rumen content (fresh and dry weight), and NDF in the rumen content. While there were no significant differences among treatments for most of the analyzed parameters, there was a tendency ($p \leq 0.10$) for the ruminal content of DM and NDF to differ between control and wood diets (mean values for the wood diet were 13.92 kg and 8.08 kg for DM and NDF, respectively; while in the control were 12.11 kg and 7.11 kg for DM and NDF, respectively.

Table.6 Effect of supplementation with wood or bark on feed intake, digestibility, milk yield and rumen contents in lactating dairy cows

	Diet least-squares mean				Probability for diet differences[3]			
Parameters	**Control**	**Wood**	**Bark**	**SED[1]**	**Diet-effect[2]**	**Control – Bark**	**Control-Wood**	**Bark - Wood**
Intake, kg/day								
Silage DMI,	12.18	6.88	6.88	0.15	0.00	<0.0001	<0.0001	0.99
Concentrate DMI	9.67	8.65	9.10	0.36	0.20	0.25	0.10	0.33
Tot. DMI	21.86	19.23	19.76	0.83	0.15	0.13	0.08	0.58
Tot. OMI	19.84	17.84	18.24	0.80	0.22	0.18	0.13	0.67
Tot. NDF	9.42	9.23	8.77	0.28	0.25	0.14	0.56	0.23
Tot. CPI	4.17	3.16	3.26	0.12	0.02	0.01	0.01	0.49
Digestibility, %								
DM	0.67	0.65	0.65	0.02	0.49	0.31	0.37	0.86
OM	0.68	0.66	0.66	0.02	0.49	0.30	0.40	0.78
NDF	0.64	0.62	0.58	0.03	0.20	0.16	0.28	0.55
CP	0.71	0.71	0.72	0.01	0.84	0.95	0.67	0.62
Milk, kg/day								
Milk	29.42	24.92	26.23	1.84	0.24	0.22	0.13	0.55
ECM	29.61	25.63	26.55	1.98	0.31	0.26	0.18	0.69
Milk fat	1.18	1.05	1.08	0.08	0.38	0.29	0.23	0.80
Milk protein	1.04	0.88	0.91	0.08	0.30	0.25	0.18	0.71
Milk lactose	1.32	1.10	1.18	0.09	0.25	0.24	0.13	0.50
Rumen, kg								
Rumen FW	84.60	84.70	77.80	4.30	0.37	0.25	0.98	0.24

Rumen DM	12.17	13.92	12.43	0.45	0.10	0.63	0.06	0.08
Rumen OM	11.11	12.65	11.29	0.56	0.18	0.78	0.10	0.13
Rumen NDF	7.11	8.08	7.13	0.26	0.10	0.95	0.06	0.06
Rumen pH	6.24	6.31	6.28	0.06	0.61	0.63	0.48	0.64

[1]Standard error of Difference

[2]Probability for F-test of diet effect

[3]Comparisonwise error probability for the difference between diets

6. Discussion

Aspen wood chips and aspen bark are by-products from the matchstick industry and aspen sawdust can serve as a potential roughage for cattle in fodder shortage situations and it can be a good alternative for hay where hay is expensive or not available (Satter et al. 1970). According to Schingoethe et al. (1981) aspen tree can be used as a roughage substitute. Singh et al. (1981) reported that aspen can substitute up to 48% of the total ration with a combination of soyabean meal as a protein source in the diet for growing steers. There has been one recent pilot study by Prestløkken et al. (2019) in Norway with the objective to investigate whether grass silage can be replaced with aspen sawdust. The authors concluded that aspen sawdust can be used as a partial roughage substitute in lactating cows. The objective of the research project reported here was to compare aspen-byproducts substituted diets with a grass-silage based control diet regarding digestibility and rumen metabolism in lactating cows. We hypothesized that 6 kg DM grass silage can be replaced with aspen by-products and that it will not affect the overall feed digestibility and rumen function of cows.

In our study, total DM intake was 21.86 kg/day for the control diet, 19.23 kg/day for the wood diet, and 19.76 kg/day for the bark diet (p= 0.08 for the difference control vs. wood diet). Schingoethe et al. (1981) reported similar DM intake for an experimental diet containing (on a DM basis) 30% aspen, 30 % corn silage, and 40 % concentrates and for a control diet with 60% corn silage plus 40% concentrate. Prestløkken et al. (2019) also recorded DM intake within the same range in late lactation cows after gradual substitution of grass silage by aspen sawdust. Their period was of 15 days and at the start of the period cows were given 6 kg DM/day grass silage and 8.7 kg DM/day concentrate and at the end of the period grass silage was reduced to 2.5 kg DM/day, while allowance of concentrate was 11.3 kg DM/day and of aspen sawdust 5.4 kg DM/day (9 kg as fed). Hence, Prestløkken et al. (2019) replaced grass silage with aspen sawdust substitution by 42 % and he found no effect on cows' health and performance, though the amount of concentrate was increased in the aspen substituted ration by 2.6 kg DM. The researcher successfully substituted grass silage by aspen sawdust, but for balancing energy intake, there was increased consumption of concentrate, which can be costly or not a good alternative in case of longer-term replacement of grass silage with aspen sawdust.

The total dry matter intake in all experimental diets showed that substituting silage with wood or bark decreased the DMI. The dry matter intake might have decreased in aspen bark and wood diets because of low aspen feed palatability. Similarly, Archibald (1926) reported that when only woody sawdust was fed to cows, cows did not eat that but when it was mixed with grains, they ate it and even those cows which had previous exposure to grain mixed with sawdust feeding, refused to eat when only sawdust was fed. It was observed in our study that when cows were only given aspen bark or wood, the cows did not eat much, we had to mix the bark or wood diet with extra basal concentrate and after that, cows started eating. But the level of total concentrate was maintained at 9.70 DM kg/day. Cows consumed numerically more basal concentrate mixed in feed troughs with bark diet, 4.35 kg DM/day as compared to wood diet 3.90 kg DM/day because more of the bark/concentrate mix was eaten than of the wood/concentrate mix. Fullinwider (1976) reported that they faced problems in feeding aspen diet to sheep and suspected that it was because of poor feed palatability. Moreover, DMI is also affected by the fiber content in the feed. Ruminal microflora needs time to act on the fiber and digest it, dry matter digestibility of structural carbohydrates is reduced with small particles size which passed out of the rumen faster without being digested but intake of fibers is increased (Chow and Russell, 1992). Similarly, Bourquin et al. (1990) pointed out that it can be two to three times reduced digestibility of structural carbohydrates as compared to non-structural carbohydrates. As the wood and bark had high fiber contents, so the DMI also decreased compared to the control diet, probably because of the rumen fill effect. Kipp (1979) suggested that for this reason, the aspen substitution rate should not be more than 30 % of total ration DM during peak lactation as it may reduce the feed intake because of the high fiber in the aspen diet. Satter et al. 1973 also recommended that 30% DM substitution of aspen diet has no effect on milk production.

The intake of NDF and OM ranked in the same way as DM intake with the largest values for the control diet, and the smallest intake for the wood diet, the bark diet being intermediate. The interesting point is that the rumen contents of DM, OM, and NDF were numerically larger in the cows fed the wood diet compared to control ($p \leq 0.10$) and bark ($p \leq 0.13$) diets. Wood has both larger fiber concentration and larger particle size than bark, which could make wood retention time longer in the rumen and hence resulting in larger NDF and DM contents in the rumen as compared to bark and control diets. Though particle sizes were not measured so it is an assumption that it might be because of different particle size as discussed in the literature review section.

Although differences were only numerical, DM and OM digestibility were only 2 percent-units less for the wood and bark diets compared to the control diet. For NDF digestibility, the span was larger, with wood diet 2 percent points less than the control and bark diet a further 4 percent points lower. The digestibility of aspen-byproduct is dependent on the level of lignin present with hemicellulose and cellulose in it. The lignin content is expected to be larger in aspen bark than in wood, which might explain lower NDF digestibility for the aspen bark diet than with the aspen wood diet. In the current experiment, the total DMI for bark was numerically larger than for wood, but NDF digestibility was less in the bark than in the wood. This effect could be due to the fact that wood contains more NDF but less lignin, as well as because of different passage rate, rumen microbiota has more time to degrade fiber in wood. Lignin is inversely related to digestibility (Kirk and Moore, 1972).

The digestion of fibers in the rumen is affected by passage rate of digesta from the rumen. The rumen passage rate depends upon different factors. Some of these are, fiber content of the diet, particle size, fiber rumen degradability, and dietary content of non-structural carbohydrates and protein. Cow digestive system is basically designed for fibrous diet and their diet must contain fibers for normal rumen function. The fibers enter in the rumen which are then digested by rumen microbes for their energy intake and rumen fermentation releases VFA's which are then digested through ruminal wall by the cow. The more fibers are in the rumen, the more is time available for the microbes to work on and hence utilizes energy. The energy gain per kg DM may decrease if passage rate goes up, but as dry matter intake can increase, so the total energy supply may still increase. Diets with low digestibility of NDF, lowers dry matter intake and ultimately total energy intake is reduced. This has been manifested by lower ruminal concentration of total VFAs as the major VFAs i.e., propionate, acetate, and butyrate, were lower in aspen rations than in control rations (Kipp, 1979; Schingoethe et al. 1981). In the current experiment, there was no significant difference in the pH of rumen, with 6.24 for the control diet, 6.31 in the wood diet and 6.28 in the bark diet. Similarly, other studies reported that there was no effect of aspen inclusion in the diet on rumen pH (Satter et al. 1970;1973; Fisher 1980; Schingoethe et al. 1981).

Wood and bark diets have more fibers than control diets and we can expect more rumen retention time than control diet and ruminating behavior might have increased as it was observed in Satter et al. (1970) experiment where substitution of aspen by-product diets have increased the ruminating time. But the recent study by Prestløkken et al. (2019) with aspen sawdust in Norway concluded that ruminating time was decreased with small size particle of aspen sawdust, and it was not affected when they introduced longer particles of aspen sawdust. So, it would be interesting to know in future studies that either aspen sawdust or its particle size has effect on the rumination time, but our experiment did not record behavior changes in the cows.

Aspen wood had larger concentration than aspen bark of NDF, while the opposite was the case for ash (table 4). Ash values were similar to what Hytönen et al. (2018) reported for hybrid aspen (*Populus tremula × P. tremuloides)* with 0.45% ash of wood DM and 3.9% of bark DM, compared to 0.63 % and 2.62 % in our experiment for wood and bark DM, respectively. This implies a somewhat larger OM concentration in the bark than in the wood. Digestibility of OM was the same for both wood and bark diet, 66%. However, the digestibility of wood and bark depends more on digestibility of their NDF (cellulose and hemicellulose and lignin) content in the feed than on the OM content. NDF digestibility further depends upon the level of lignification (lignin level) in the diet (McDonald, 2010) and it can be assumed that bark has more lignin content than wood diet. Kipp (1979) reported that aspen wood contains 16.9% lignin of DM as compared to aspen bark which contains 23.6% (Enzmann et al. 1969) and 22.6% (Yemele et al. 2008).

One concern about feeding aspen by-products is that it can have an effect on the milk production/composition due to its low content of CP (less than 2%). In the current study, we maintained the CP content of all three diets by concentrates and protein supplements. Yet, CP contents were lower (16.43% for wood diet, 16.49% for bark diet) than in the control diet (19.07%) but consistent with the studies done by Olmos et al. (2006) in which 16.5% CP of ration DM was optimal for cow's milk production. Regarding experimental design and compared to continuous treatment trials, change over designs can have carry-over effects. Our study was a changeover trial with three cows in a Latin square arrangement. It can´t be ruled out that individual cows had different adaptability towards the different diets and, moreover, towards the sequence the different treatments were imposed. For example, cow first eating wood, then bark and ultimately given control diet may not behave similar as cow which gets control diet first followed by wood and bark diets. A bigger study involving a higher number of animals including extra periods to account for the possible carry-over effects would be needed, before we can finally comment whether substitution with aspen by-products has positive or negative effect or even if there is no effect at all, on cow health and production. There had not been many trials conducted to study the effect of inclusion of aspen by-products in diets for high producing lactating cows, so very limited literature is available.

7. Conclusions

Results of the present study suggest that aspen by-products could be used as a source of roughage, by partly substituting grass silage in diets for lactating dairy cows, without any significant detrimental effect on rumen function and milk yield and composition.

However, as aspen by-products have a very low content of CP, extra care should be taken to supplement CP while substituting in the roughage's diets of high producing lactating cows.

Moreover, more studies involving a larger number of animals are needed before to recommend suitable levels of inclusion of aspen by-products on commercial dairy farms.

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