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Granulated Animal Feed and Fuel Based on Sea Buckthorn Agro-Waste Biomass for Sustainable Berry Production

Anna Andersone [1,2,*], Sarmite Janceva [1,*], Liga Lauberte [3], Natalija Zaharova [1,2], Mihail Chervenkov [4,5], Vilhelmine Jurkjane [1], Lilija Jashina [1], Gints Rieksts [1,6] and Galina Telysheva [1,†]

1 Latvian State Institute of Wood Chemistry, Dzerbenes 27, LV-1006 Riga, Latvia; natalija.zaharova@gmail.com (N.Z.); telysheva@gmail.com (G.T.)
2 Ekokompozit Ltd., Dzerbenes 27, LV-1006 Riga, Latvia
3 Laboratory of Finished Dosage Forms, Riga Stradins University, Dzirciema 16, LV-1007 Riga, Latvia; liga.lauberte@rsu.lv
4 Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria; vdmchervenkov@abv.bg
5 Faculty of Veterinary Medicine, University of Forestry, 1756 Sofia, Bulgaria
6 The Institute of Physics, University of Latvia, Miera 32, LV-2169 Salaspils, Latvia
* Correspondence: anna.andersone@kki.lv (A.A.); sarmite.janceva@kki.lv (S.J.); Tel.: +371-29104319 (A.A.); +371-25148850 (S.J.)
† The article authors from the Latvian State Institute of Wood Chemistry and Riga Stradinš University are grateful to Professor, Dr. habil. chem. Galina Telysheva, who started the work with sea buckthorn in this project and was our great leader until her passing in November 2021, for teaching and inspiring us to study a unique material—lignocellulosic biomass.

Abstract: The industrial harvesting of sea buckthorn (SBT) berries with twigs and subsequent pruning creates a large volume of lignocellulosic agro-waste. This study aimed to valorize this agro-waste as a raw material for animal feed and fuel granules, for developing a sustainable cascading SBT production scheme. Five SBT cultivars' biomasses were characterized by analytical pyrolysis, mass spectrometry, and GC analysis. Condensed tannins, which are undesirable components for animal feed, were separated by extraction. The residue was analyzed for total protein, vitamins (A, C, and E), ash, crude fat, wood fiber, and macroelements (P, K, Ca, and Na), and showed great potential. The heavy metal (Cd, Hg, and Pb) content did not exceed the permitted EU maximum. Granulation regimes were elaborated using a flat-die pelletizer, KAHL 14-175. The digestibility and the amount of produced gas emissions were determined using in vitro systems that recreate the digestion of small ruminants. The investigation proved that SBT leaves and stems are a unique underutilized source of animal feed, used alone or in combination with others. Twigs, due to their thorns, were granulated and valorized according to standards for application as fuel. The scheme offered in this study enables SBT agro-waste utilization and sustainable SBT berry production.

Keywords: sea buckthorn; agro-waste; lignocellulosic biomass; condensed tannins; animal feed; digestability; greenhouse gas emissions; small ruminants; granulated feed; granulated fuel

Citation: Andersone, A.; Janceva, S.; Lauberte, L.; Zaharova, N.; Chervenkov, M.; Jurkjane, V.; Jashina, L.; Rieksts, G.; Telysheva, G. Granulated Animal Feed and Fuel Based on Sea Buckthorn Agro-Waste Biomass for Sustainable Berry Production. *Sustainability* **2023**, *15*, 11152. https://doi.org/10.3390/su151411152

Academic Editor: Igor Palčić

Received: 6 June 2023
Revised: 10 July 2023
Accepted: 13 July 2023
Published: 17 July 2023

1. Introduction

The world population is rapidly increasing: it is expected that there will be nearly 10 billion people on Earth by 2050 [1]. Agricultural production grows accordingly [2], and sustainability is the only way for mankind to survive, minimize negative effects on the environment, and keep the planet's population healthy [3].

Animal farming demands 70% of agricultural land and 30% of the earth's land surface [4–6]. An increase of 50% in animal feed is necessary by the year 2050; thus, to prevent the expansion of pasture areas with more than 500 mln hectares (hm^2) [1] and save the forests, it is extremely important to study the huge amount of agricultural waste and use it in the best way possible. Agro-waste, as a source of animal feed and feed

additives, could be one of the possible ways to sustainable agriculture. Feed accounts for 60–70% of total expenses for livestock and poultry [7]. Insufficient amounts of raw materials and growing costs have led to an imbalance in the animals' diets and a decrease in zootechnical indicators.

People's growing interest in a vegetarian diet nevertheless supports the demand for milk products. If everyone were vegan, the land use for agriculture would decrease by 75% [8]. However, it is highly unlikely to happen as vegans today comprise a mere 1–2% of the world population [9,10]. The problem of waste-free production is real one in any case.

Hippophae rhamnoides L. (sea buckthorn, SBT) of the family *Elaeagnaceae* is a unique fruiting shrub tree that can survive in extreme temperatures (from –43 °C to 55 °C) and grows well under drought conditions. According to legend, people in ancient Greece discovered the plant and its benefits in feeding racehorses [11]. Today, the industrial economically viable harvesting of SBT berries is possible only by cutting the whole berried branch. An SBT plantation can yield 25 tons of berries from 1 hm^2 bi-yearly, which is 12.5 tons per year and hm^2 [12]. Waste lignocellulosic biomass amounts to around 20–30% of the berries' mass. Foliage yield from the whole SBT tree could reach 16 tons/ha which is more than from any shrubs and grasses [13]. Moreover, berry-producing trees must be cut every four years, otherwise, berries will be difficult to harvest. SBT trees that are used for land reclamation and the improvement of soil quality, thanks to their roots' nitrogen-fixing ability (an SBT plantation with 8–10-year-old trees is able to fix nitrogen in the amount of 180 kg/hm^2 in a year [14]), also have to be pruned. As a result, lignocellulosic biomass, containing twigs, stems, leaves and even roots, is appearing as agro-waste in large amounts. SBT grows in 52 countries, on a total area of 3 mln hm^2, according to a 2023 report [15]. The SBT berry has a high nutritional and pharmaceutical value [11,15–22]; however, its economic potential is still underdeveloped due to the high expenses of SBT berry harvesting [23]. The human labor expenses for SBT harvesting were determined to be 58% of the total production costs [24,25]. Therefore, the application of side streams is necessary both for sustainability and economic feasibility. "Fodder trees" can be considered as new multipurpose solutions since the side-product—agro-waste—grows in the same cultivation area and does not demand new agricultural lands for producing feed [26,27].

Currently, SBT wood residues (twigs and stems) are mainly used as a renewable energy source. A 6-year-old SBT orchard can provide tons of fuel wood, whereas, one ton of SBT wood is equal to 0.68 tons of conventional coal [28]. Out of the total SBT biomass, leaves are valorized for other applications in some countries. In China, Mongolia, Scandinavian countries, Germany, the Czech Republic, Latvia, Russia, and Greece, SBT leaves are used for the preparation of tea with antioxidant properties [11,29–31]. In India, leaves are used as a feed additive for chicken and cattle [32], and in Mongolia—for the treatment of colitis and enterocolitis in humans and animals [33]. It was found that SBT leaves are the richest source of protein compared to other tree leaves [34], and SBT berries have a stimulating influence on the growth, immunity, and production performance of poultry and livestock without toxicity effects [17,35]. The body weight and egg production of chicken increased greatly after being supplemented with SBT leaves, seeds, and fruit residues [13]; however, further research is necessary in this area [36,37]. SBT pomace can be added successfully to the diet of ram lambs as well [38]. No toxic or carcinogenic side effects of berry-based products were reported [18]. There are some commercially available animal digestive supplements based on berries [39,40]. However, the research on the SBT agro-waste biomass profile of bioactive components and research on its influence on ruminants is still very limited [19,36]. There are no data available on small ruminant feed based on SBT lignocellulosic biomass, and there is no commercial animal feed production based on SBT agro-waste biomass.

Condensed tannins (CTs), except when in small concentrations, could have a negative effect on animals' digestibility [41]. Moreover, animals may not like their bitter taste. Therefore, they could be isolated and, as shown in previous studies, could find applications in health care, cosmetics, and food industries due to their anti-inflammatory and anti-bacterial properties [42].

Measurements of gas production could help to find correct feed compositions to minimize the negative greenhouse gas (GHG) impact on the environment. Digestive health remains one of the key factors in the high productivity of farm animals. The leaves and stems of SBT can be compared with grass in terms of a wide range of biologically active compounds and nutrients; thus, they can be used as a valuable food source for animals during winter. Twigs that have sharp thorns which are dangerous for animals even after grinding could be investigated for granulated fuel production. The densification of SBT biomass residues by granulation after CT extraction enables better transportation, dosing, and storage properties.

The objective of this study was the evaluation of SBT agro-waste as a raw material to obtain granulated animal feed and fuel granules for the development of a sustainable cascading production scheme in SBT cultivation (Figure 1).

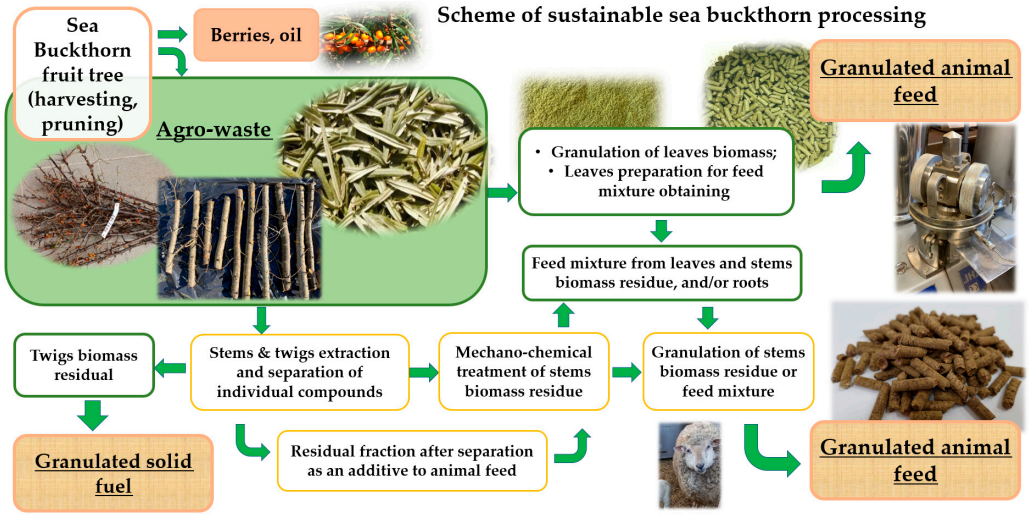

Figure 1. Scheme of sustainable sea buckthorn processing with the production of animal feed.

Such a scheme for cascading sustainable SBT processing, as well as experimental work on nutritional values, digestibility, and feed/fuel granule compositions, are novelties of the present research: all vegetative parts of the SBT tree (stem, leaves, twigs, and roots) were evaluated for the production of granulated feed for small ruminants; twig extracts were studied for the production of feed functional ingredients with antimicrobial properties; and extraction residues were evaluated as granulated fuel.

The development of alternative feed and feed additives on the market is also necessary to optimize livestock feeding costs and to provide a backup supply chain in times of economic instability and fluctuating energy prices. Building berry-producing plantations requires considerable investment [12]—and, thus, innovative agro-waste utilization turning it into valuable products is necessary.

2. Materials and Methods

2.1. Collection of Agro-Waste Biomass

The stems (ST), twigs (TW), leaves (LV), and roots (R) comprising the agro-waste biomass (further in the text—biomass) of SBT cultivars 'Maria Bruvele' (MB), 'Botanicheskaya Lubitelskaya' (BL), 'Tatiana' (TAT), 'Tarmo' (TM), and 'Otto' (OT) were collected from the four-year-old trees of an SBT plantation area in Latvia (80 trees of each cultivar) during the summer of 2021. The trees grew on the same land, and they were treated the same. The biomass was dried at room temperature. A knife mill, Retsch SM100 (Retsch, Haan, Germany), was used for grinding, and the LV particle size after grinding was 1–2 mm, and those of ST, TW, and R—2–4 mm.

2.2. Isolation of the Condensed Tannins from the ST and TW Biomass

An ethanol–water solution (80:20 vol.% ethanol:water solution, further in the text—80% EtOH) was used for the biomass extraction followed by CT isolation from the extract; the temperature of the extraction solution was 60–70 °C, and the mass ratio of biomass sample to 80% EtOH = 1:8, *w*/*w*. CT separation from the extract was carried out using a Sephadex LH-20 as described in Janceva et al. [42]. Confidence interval: CI $\leq$ 0.5% at α = 0.05.

2.3. Mechanochemical Treatment of SBT Biomass

The mechanochemical treatment of SBT initial ST biomass and ST biomass samples after CT extraction was carried out for evaluation of its effect on digestibility. Mechanochemical treatment was carried out separately for each sample in an original trituration-type mill (original construction, Riga, Latvia). The trituration of ST was carried out for 20 min, at 100 rpm.

2.4. Initial and Treated Biomass Characterization

All determinations are expressed on a dry matter (DM) basis (moisture content of initial and treated biomass less than 1%).

2.4.1. Crude Fiber Content Determination

The content of crude fiber in SBT biomass samples was determined gravimetrically by acid hydrolysis with H_2SO_4 (1.25%, *w*/*v*), used for the extraction of sugars and starch, followed by alkaline hydrolysis with NaOH (1.25%, *w*/*v*) which removes proteins, some hemicellulose, and lignin, as described by Joslyn et al. [43]. The weight of the biomass sample for one analysis was 20 g of DM. Each experiment was performed in triplicate. CI $\leq$ 0.3% at α = 0.05.

2.4.2. Total Protein Content Determination

The Kjeldahl method was applied for the determination of the total protein content in the SBT biomass [44]; a sample of 2 g was taken for analysis, and an appropriate nitrogen factor (NF—6.25) was used for the estimation of the total protein content. Each experiment was performed in triplicate. CI $\leq$ 0.3% at α = 0.05.

2.4.3. Determination of Crude Fat, Crude Ash, Macro-Elements, and Heavy Metal Content

To ascertain the content of crude fat, the extraction of fat from the SBT biomass samples by hexane was used. The weight of the obtained fat was measured, and the content of fat was expressed in % of the weight of the biomass sample. A sample of 10 g was taken for each analysis. The crude ash content (sample of 5 g for each experiment) was determined after biomass sample ignition at 550 °C in a Carbolite ELF 11/6B furnace while measuring the weight of the residue. The content of ash was expressed as % of the weight of the biomass sample. Organic matter content was calculated as the difference between the dry biomass content (taken as 100%) and the content of ash in %.

The contents of the macroelements and heavy metals were determined by ICP-MS analysis using a Thermo Fisher Scientific iCAP TQe (Bremen, Germany) fitted with a nebulizer, a quartz spray chamber, with a sampling cone made of nickel, and a skimmer cone with platinum tip, as described in Naccarato et al. [45]. A peristaltic pump and an autosampler ASX-560 (both from Thermo Fisher Scientific, GmbH, Bremen, Germany) were used to pump the solutions from the tubes. Following a 20 to 30 min period of ICP-MS stabilization, the working capacity was adjusted before the analyses to maximize the signal and minimize interference effects by applying a tuning solution based on the torch's horizontal and vertical location, the extraction lens, and the CCT (collision cell technology) focus lens. The highest purity argon and helium gas (99.99%) was employed as the carrier gas at 0.8 mL/min in auxiliary flow, at 1.0 mL/min, and 5.3 mL/min in nebulizer flow. Nitric acid (65%), Suprapur® for the trace analysis (Supelco), and hydrogen peroxide (30%) were all used in the sample digestion process. Calibration curves for quantitative analysis

were elaborated with the diluting of multielement solutions (10 mg/L); Cd, Ca, Pb, K, and Na (10 mg/L, Merck, Germany); and Hg element solution (1000 mg/L, Merck, Germany). The calibration standards, the procedure blanks, and the samples made up each batch of analysis. The weight of each sample was 100 mg. The majority of the elements under investigation were examined in kinetic energy discrimination mode (KED-mode) at the operational helium gas collision cell. Each experiment was performed in triplicate.

CI for crude fat: CI $\leq$ 0.6% at α = 0.05; for ash and organic matter: CI $\leq$ 0.9% at α = 0.05.

CI for heavy metal content is given in the Results Section 3.5, under corresponsing Table.

2.4.4. Determination of The Total Amount of Carbohydrates

Gas chromatography (GC) analysis before and after hydrolysis, reduction, and acetylation was used to determine the total amount of carbohydrates in the extracts, as well as their composition. For each experiment, a 10 mg sample was used. The analysis was performed using a GC System of the Agilent 6850 Series (Agilent Technologies, Inc., Santa Clara, CA, USA) as described in Blakeney et al. [46]. A DB-1701 column was used (the length of the column: 30 m; internal diameter: 0.25 mm; layer thickness: 0.25 µm). The analysis was repeated 3 times for each sample. CI $\leq$ 0.8% at α = 0.05.

2.4.5. Determination of Vitamin Content

The content of vitamin C (ascorbic acid) in the SBT biomass was determined by high-performance liquid chromatography (HPLC) as described in Ciulu et al. [47]. An HPLC-UV-Vis/-RI system (high-performance liquid chromatograph with UV–vis and RI detector) was used (Vanquish CORE, Dionex Softron GmbH, Part of Thermo Fisher Scientific, Germering, Germany). The extracts were rapidly dissolved in a purified water mixture of 2 M NaOH and 1 M phosphate buffer. Separation was performed on an Eclipse XDB-C18 Zorbax column (5 µm, 150 cm $\times$ 0.46 cm i.d., Agilent); the column was heated to 35 °C, and as a mobile phase, trifluoroacetic acid aqueous solution (0.025%, *v*/*v*) (A) and acetonitrile (B) were used. The gradient elution was applied (100% to 60% A in 20 min), at a flow rate of 1 mL/min. The injection volume was 20 µL. The UV detector settings: 254 nm.

The content of vitamin E as α-tocopherols and vitamin A as retinol in the biomass were determined by HPLC analysis, as described by Sibel Konyalıoğlu et al. [48] using an HPLC-UV-Vis/-RI system (Dionex Softron GmbH, Part of Thermo Fisher Scientific, Germering, Germany). A Hichrom 5 C18 column (25 cm $\times$ 4.6 mm i.d.) was used; methanol was used at the mobile phase, and the flow rate was 2 mL/min. The column was heated to 40 °C. The dry extracts were dissolved in methanol. Ten microliters of each aliquot were injected into the HPLC column. Detection was at 292 nm. Each experiment was performed in triplicate. The CI is given in the Results Section 3.5, under corresponsing Table.

2.4.6. Analytical Pyrolysis

The analytical pyrolysis (Py-GC/MC/FID) method was applied for the chemical characterization of SBT biomass. The temperature of pyrolysis was 500 °C and the heating rate was 600 °C/s. A Frontier Lab Micro Double-shot Pyrolyzer Py-3030D directly coupled with a Shimadzu gas chromatograph GC/MS/FID-QP ULTRA 2010 (Fukushima, Japan) was used. The capillary column was RTX-1701 (Restec, Metairie, Louisiana, USA), 60 m $\times$ 0.25 mm $\times$ 0.25 mm film. The injector temperature was 250 °C; ion source with EI of 70 eV. The MS scan range was 15–350 m/z. Helium was used as a carrier gas, the flow rate was 1 mL/min, and the split ratio was 1:30. A sample of 1.20 mg was taken for each analysis. The individual compounds were identified by GC/MS with the help of library MS NIST 11 and NIST 11s. On the basis of GC/FID data, the relative peak areas for the individual compounds were calculated using Shimadzu software. The relevant peaks' summed molar areas were normalized to 100%. The pyrolysis analysis was repeated four times and the data were averaged. The variation coefficient of measurement was $\leq$5%.

2.4.7. Elemental Analysis

The elemental composition (C, H, and N) of the SBT biomass samples was determined using a Vario MACRO CHNS elemental analyzer with a heat conduction detector (Elementar Analysensysteme GmbH, Langenselbold, Germany). The dry sample was weighed in a foil (weight of sample: 50 mg DM). The WO_2 powder was used as a combustion catalyst, in a ratio of 1:1 (w/w). The obtained sample/catalyst mixture was pressed into a tablet and placed in the automatic sample feeder (carousel). The equipment was controlled in a computerized mode and VARIOEL V5.16.10 software was used for data processing. The results were expressed as percentages of DM. Three repetitive analyses were performed for each sample. CI $\leq$ 0.2% at α = 0.05.

2.5. Preparation of Animal Feed Compositions

Stem (ST) biomass in compositions of animal feed was used as the residual fraction after extraction and CT separation and after mechanochemical pre-treatment (MT; mechanochemically treated stems further in the text—ST/MT). Leaves (LV), roots (RT), ST/MT, and mixes of LV with ST/MT were investigated as feed additives. The ratios of ST, LV, and RT used for analysis were as follows: LV 100%, ST 100%, ST/MT 100%, LV:ST/MT (1:1; w/w), and LV:ST (1:1; w/w). In addition, for the granulation experiment, the mix with roots was used, LV: ST/MT (1:1, w/w) + 5% of roots.

2.6. Determination of Released Gas Emissions, In Vitro Analysis

The amount of the in vitro gas production (GP) was determined using an ANKOM RF Gas Production System (AGPS; ANKOM Technology, Macedon, NY, USA), which is designed for analysis of different feed sources and feed additives. The in vitro gas production method is based on the relationship between the fermentation in the rumen and the gases formed and can also be used to measure and quantify nutrient utilization. Rumen fluid was collected from slaughterhouse animals (rams) following the protocol of Fortina et al. (2022) [49], with small modifications. It was decided to use rumen fluid collected from slaughterhouse animals, first, because a significant difference in in vitro digestibility has not been found when the fluid was obtained from slaughtered or fistulated ruminants [50], and, second, because this was a more ethically acceptable approach [51]. It was reported that it is possible to store the rumen fluid without significant quality changes by putting it in thermic bottles wrapped in a thermic bag, for a period of up to 300 min after collection [49]. The methodology and test conditions were in accordance with the prescriptions provided by the manufacturer and following the protocol of Videv [52]. In short, a feed sample in the amount of 0.500 $\pm$ 0.001 g, 25 mL of rumen fluid, and 50 mL of incubation medium, made as described by Theodorou et al. [53], were placed in each of the modules of the system. Each of the 50 modules of the ANKOM system has pressure measuring sensors installed (the range is: $-$69 to 3.447 kPa; resolution: 0.27 kPa; accuracy: $\pm$ 0.1% of the measured value). Specialized software received the data from every module through a wireless connection and recorded it every 30 s. GP was expressed as mL/g incubated DM. The changes in gas pressure accumulated during 24 and 48 h of fermentation (ΔP) were converted into volume units by applying the ideal gas law:

$$\text{GP (mL/g DM)} = (\Delta P/Po) \times Vo, \text{(mL/g incubated DM)}, \quad (1)$$

where ΔP is the change in the accumulated pressure (expressed in kPa) at the top of the module, Vo is the volume of the bottle at the top (235 mL), and Po is the atmospheric pressure which was recorded by the apparatus before the beginning of the experiment.

For taking into account the final volume of released gas from the rumen fluid itself, a blank module without a sample of the feed was used. The zero module, placed above the incubator, took into account the atmospheric pressure in the room and corrected the data according to atmospheric pressure. The samples were analyzed in triplicate.

2.7. Determination of Digestibility of the SBT Biomass Samples

For the evaluation of digestibility, an Ankom Daisy incubator was used (Ankom Technology, Macedon, NY, USA). The rumen fluid, used in this testing, was collected as described in Section 2.6. The methodology and test conditions were in accordance with the manufacturer's prescriptions and followed the protocol of Kiliç et al. [54]. Four rotating digestion jars (or cylinders) were placed in the specially designed and controlled Daisy incubator where a constant, uniform heat (≈39 °C) was maintained and agitation was provided. A buffer solution (1600 mL) and rumen fluid (400 mL) were used as inoculums for each cylinder. The dry samples in the amount of approximately 500 mg were placed in filter bags (25 pcs). Then, the filter bags were placed into the cylinders with the inoculum. Aeration of the cylinder was performed for 30 s using CO_2, and then the cylinders were tightly closed (immediately after aeration) and placed in the incubator for 48 h. After 48 h of incubation, the filter bags were taken out from the incubator. Water was used for cleaning the bags, and then the bags were dried at 105 °C, for 3 h. Analysis for neutral detergent fiber (NDF) digestibility of the contents of the bags was performed with a fiber analyzer. In vitro true digestibility was calculated according to the following equation:

$$\text{IVTD, \%} = 100 - ((\text{W3} - (\text{W1xC1})) \times 100)/\text{W2} \quad (2)$$

where IVTD is the in vitro true digestibility of feed, W1 is the weight of the filter bag, W2 is the weight of the sample put in the bag, W3 is the weight of the bag with the sample after NDF analysis, and C1 is the correction coefficient for the weight of the bag without a sample.

Three repetitions of the in vitro experiment were performed. The confidence intervals are given in the Results Section 3.7.

2.8. Antimicrobial Activity of the SBT Fraction

Analysis of the antimicrobial activity of the residual fraction after CT separation from the extracts of both stems and twigs (MB/ST+TW) was performed at the University of Latvia, Faculty of Biology. Several reference microbial strains were used, which were obtained from the Latvian Microbial Strain Collection (MSCL), University of Latvia: *Pseudomonas aeruginosa* MSCL 3314, *Staphylococcus aureus* MSCL 3340, *Escherichia coli* MSCL 332, *Bacillus cereus* MSCL 330, and *Candida albicans* MSCL 378. Antimicrobial activity was analyzed in 96-well plates by the two-fold serial broth microdilution method [55]. As a result, the values of the minimum inhibitory (MIC) and minimum bactericidal/fungicidal concentrations were ascertained (MBC/MFC). CI $\leq$ 0.01 at α = 0.05.

2.9. SBT Biomass Granulation and Characterization of the Pellets

2.9.1. Biomass Granulation

For the simulation of the pelletizing in real production conditions, a laboratory pelletizer KAHL 14-175 (Amandus Kahl GmbH & Co. KG, Reinbek, Germany) equipped with a flat die, analogous to the factory-scale KAHL granulators, was used for granulation. The channel diameter was 6 mm, and the channel length-to-diameter ratio was 4:1. Each experiment was performed in triplicate. Preliminary granulation of sawdust until the equilibrium temperature in the pelletizer reached 50 °C was performed. The weight of the studied biomass used for one granulation experiment was 2 kg.

2.9.2. Characterization of the Pellets

The measurement of the ash content was performed, expressed as a % of the weight of the residue after the ignition of solid biomass samples at 550 °C in a muffle furnace to the initial weight of the solid biomass sample, according to the EN ISO 18122:2023 standard [56]. Higher heating value (HHV) was determined experimentally by burning granules in a calorific bomb of original construction (Li-104, Latvia), according to the standard ISO 18125:2017 (solid biofuels) [57] and calculated on a DM basis. A sieve with a 3.15 mm cell

size was used for the separation of fines (ISO 3310) [58]. After the fines separation, the determination of the durability (DU) and bulk density (BD) was performed based on the European standards EN ISO 17831-1:2016 [59] and ISO 17828 [60], correspondingly. The CI for the HHV and LHV of the pellets: ±0.6 MJ/kg; the CI for durability: ±0.7%; the CI for bulk density: ±11 kg/m^3; the CI for ash content: ±0.6%; the CI for the average length of pellets: ±10 mm.

2.10. Statistical Analysis

All experiments were conducted in triplicate, except for analytical pyrolysis (Py-GC/MS/FID) and gas chromatography (GC/MS/FID) analysis where four repetitive experiments were performed. The results are expressed as means. Microsoft Excel 2016 was used for statistical analyses. Confidence intervals (CI) were calculated for a mean using Student's *t*-distribution, and a significance level of 5% was applied ($\alpha = 0.05$). For the evaluation of the strength of the linear relationship between two different variables, Pearson's correlation coefficient was calculated. A significance level of $p < 0.05$ was applied.

3. Results and Discussion

3.1. Chemical Composition of SBT Biomass

3.1.1. Organic Matter in the Biomass

It was found that the biomass samples had a high content of organic matter (95.5–98.1%/DM), which included such components as proteins, fats, fiber, and non-structural carbohydrates (sugar and starch). The ash content of the SBT biomass samples ranged from 1.9 to 5.2%/DM. The highest ash content was in leaves (4.8–5.2%/DM) and roots (4.0–4.5%/DM).

3.1.2. Relative Composition of SBT Biomass by Py-GC/MS/FID

According to the results of analytical pyrolysis analysis, the main components of organic volatile products of SBT biomass DM are carbohydrates, including low-molecular-weight sugars, starch, and various non-starch polysaccharides, which are the most important sources of energy for non-ruminants and ruminants. The total carbohydrates-derived volatile contents in the SBT stems, leaves, and roots were 66.7–68.4% rel, 56.7–60.6% rel, and 72.8–75.9% rel/TVP, respectively (Figure 2).

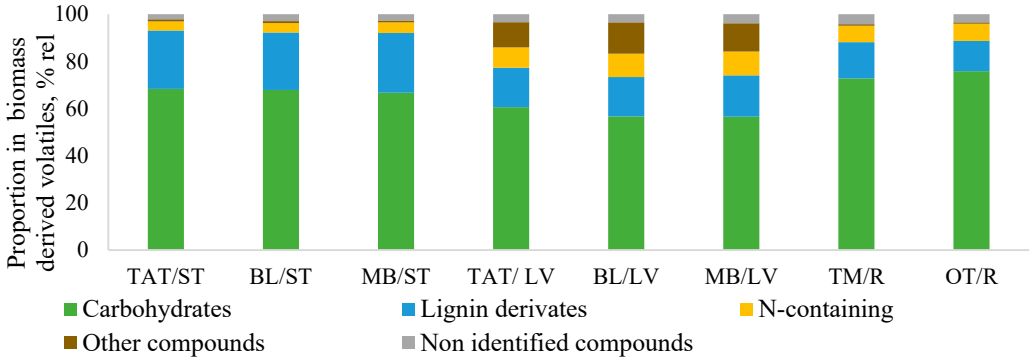

Figure 2. Py-GC/MS/FID data of organic volatile products of SBT biomass: TAT—Tatiana; BL—Botanicheskaya Lubitelskaya; MB—Maria Bruvele; OT—Otto; TM—Tarmo; ST—stem; LV—leaves; R—roots.

The carbohydrate concentration of the SBT roots was 1.1 and 1.3 times higher in comparison to the stems and leaves biomasses, respectively.

3.1.3. Carbohydrate Composition by GC-MS

Based on the results of gas chromatography analysis, the main sugar monomer units of roots' carbohydrate composition were glucose (73.6–77.1%/total carbohydrate content of root DM) and mannose (10.1–12.9%/total carbohydrate content of root DM). The total

contents of galactose, xylose, and arabinose were 2.1–3.1%, 3.2–4.6%, and 6.5–6.8%/total carbohydrate content of root DM, respectively (Figure 3).

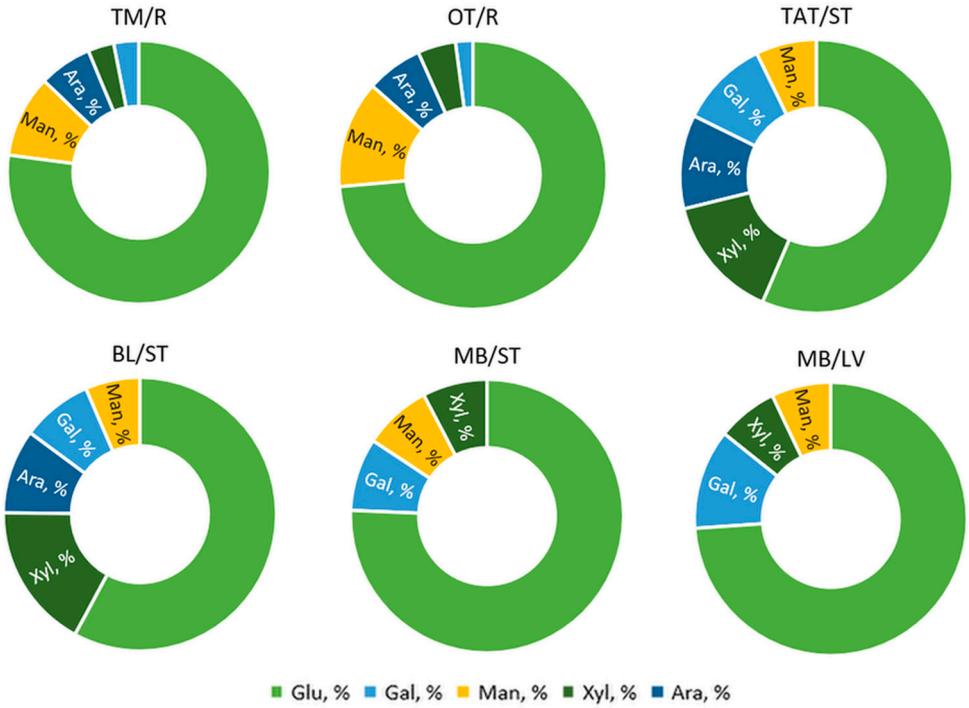

Figure 3. The sugar composition in total carbohydrates of SBT biomass (GC data): TAT—Tatiana; BL—Botanicheskaya Lubitelskaya; MB—Maria Bruvele; OT—Otto; TM—Tarmo; ST—stem; LV—leaves; R—roots.

The amount of glucose in the composition of total identified sugars of the SBT stems, roots, and leaves was close for each vegetative part between the five cultivars. The amount of xylose was the highest for the stems of BL and TAT. Xylose is not a desirable component in feed: it was proven that in high amounts it could reduce ruminal digestibility of various animal feeds [61,62]. Therefore, for the subsequent first experiments, stem samples of MB with comparatively smaller content of xylose were chosen. The structural (free) carbohydrate (monosaccharide) content in SBT biomass did not exceed 1%/DM. To make carbohydrates more available as an energy source, pretreatment was considered. It was reported that it is possible to increase the surface area of cellulose up to 10^6 times by decreasing its particle size [63], and thus improve the nonmotile cellulolytic microbe penetration into the cell lumen [64].

3.1.4. Relative Composition of SBT Biomass Phenol/Lignin Part by Py-GC/MS/FID

The total phenol/lignin-derived volatile (Ph/L-DV) contents in the SBT stems, leaves, and roots were 24.4–25.4% rel, 16.7–17.6% rel, and 12.8–15.3% rel/TVP, respectively. The phenol/lignin-derived pyrolysis products can be divided into phenyl (Ph) and benzyl (B), guaiacyl (G), and syringyl (S) derivatives in SBT biomass. The Ph/L-DV of SBT stems have the highest content of G derivative units (43.8–50.3% rel/Ph/L-DV), and fewer S (32.0–35.0%/ rel Ph/L-DV) and Ph and B units (17.4–21.2% rel/Ph/L-DV). The Ph/L-DV of leaves and roots presented more Ph and B derivative units, 40.8–44.9% and 69.7–71.9%, respectively (Figure 4).

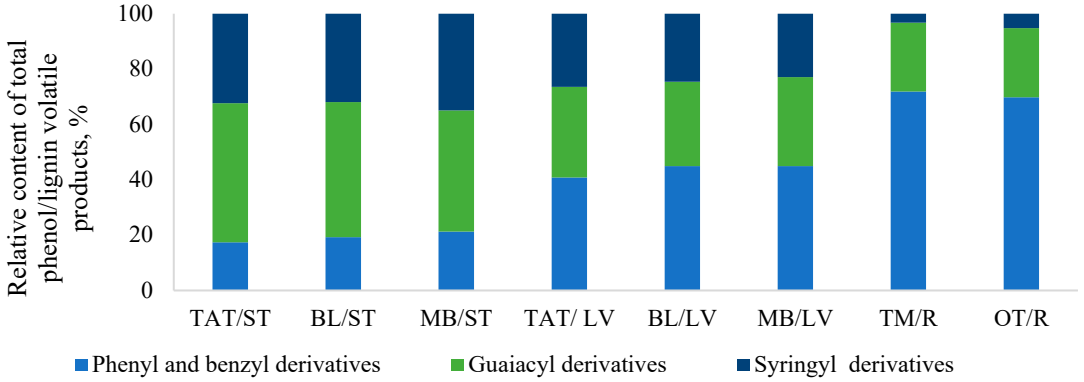

Figure 4. Relative contents (%) of phenyl and benzyl, guaiacyl, and syringyl derivatives in the Ph/L-DV released after Py-GC/MS of SBT biomass: TAT—Tatiana; BL—Botanicheskaya Lubitelskaya; MB—Maria Bruvele; OT—Otto; TM—Tarmo; ST—stem; LV—leaves; R—roots.

The phenyl and benzyl derivatives came from polyphenolic compounds that have antioxidant activity [65] and could serve for the oxidative stability of animal feed. Since phenolic compounds will remain in the residual biomass fraction after the separation of CTs, their antibacterial activity will be tested (Section 3.1.2). Lignin is a hardly digestible source and its strong bonds in lignin–carbohydrate complexes are the main obstacle to wood-containing part application in animal feed [66]. Therefore, mechanochemical pre-treatment was further investigated in this study (Section 3.3) for the possibility of degrading the cell wall.

3.2. CT Separation

According to the literature data, CTs, which are found among polyphenolic compounds in SBT biomass, are also anti-nutritional since they bind proteins. However, CTs are strong antioxidant and antimicrobial agents and can be used in cosmetics, the production of adhesives, and other related industries [19,67]. Among the studied biomass samples (stems, twigs, roots, and leaves) only SBT stems and twigs contained CTs in an amount of 6 to 11%/DM. Only the stems could be used for animal feed production since the twigs have sharp thorns, and therefore twigs will be tested for granulated fuel production. However, CT, as a valuable compound, was preliminarily isolated from both stems and twigs. The correctly chosen extractant made it possible to completely remove CTs from the stem and twig biomasses.

3.3. Mechanochemical Pre-Treatment for the Improvement of Digestibility

In animal nutrition, lignin cannot be readily fermented by rumen microbes. The solution to this was the use of mechanochemical processing. The mechanochemical treatment disrupts the cell wall of the plant, thereby facilitating the digestibility of valuable components. The digestibility results of SBT stems after CT separation before and after mechanochemical processing are shown in 3.7.

3.4. The Anti-Microbial Properties of the Residual Fraction after CT Separation

The residual fraction after CT separation contained serotonin, low-molecular-weight polyphenolic compounds (quinic acid, catechin, etc.), and their glycosides [19]. In this study, this residual fraction's antimicrobial activity was evaluated against Gram-positive and Gram-negative bacteria as well as pathogenic fungi. The lowest MIC/MBC values for the fraction were the following: 0.78/0.78 mg/mL against *E. coli*, 1.56/3.13 mg/mL against *P. aeruginosa*, 1.56/>50 mg/mL against *B. cereus*, and 0.78/1.56 mg/mL against *S. aureus*. The lowest MIC/MFC against *C. albicans* was 12.50/>50 mg/mL. This showed that enriching the biomass with the above-mentioned low-molecular-weight components and

returning them to the biomass is a way to create feed additives with special antimicrobial target properties (Figure 5).

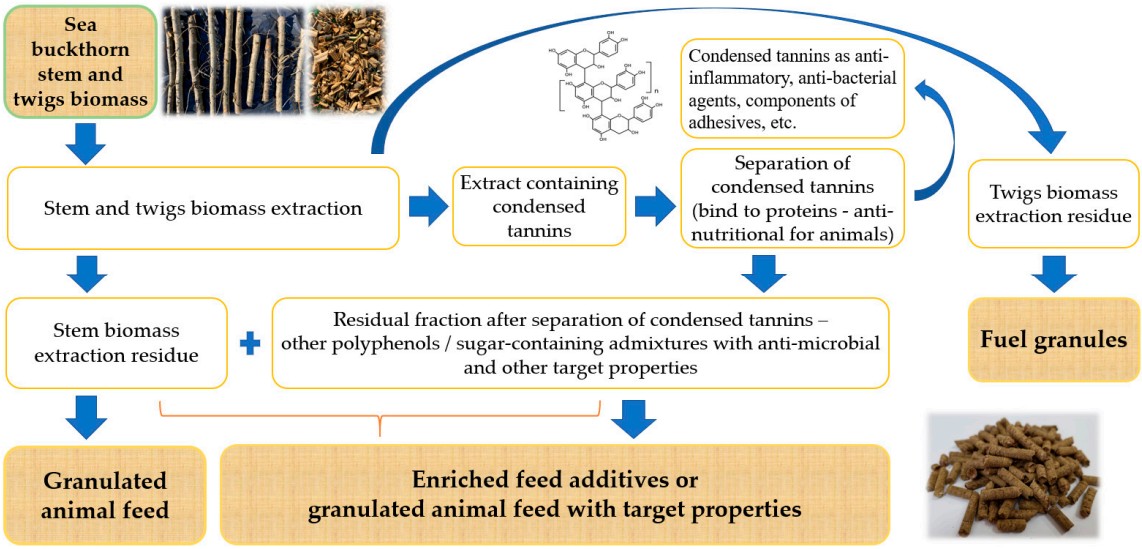

Figure 5. SBT stems and twigs application scheme.

3.5. Macro-Nutrients and Vitamins in SBT Biomass

The physiological and functional processes of an animal are influenced not only by organic matter but also by the inorganic components in the feed additive. The results of the analyses are shown below in Table 1.

Table 1. Contents of macronutrients and heavy metals in SBT biomass.

Macronutrients and Heavy Metals *	MB/LV	MB/ST	BL/LV	BL/ST	TAT/LV	TAT/ST
P, mg/100 g DM	225 ± 22	220 ± 22	210 ± 21	199 ± 20	212 ± 18	217 ± 26
K, mg/100 g DM	1376 ± 113	1109 ± 107	1209 ± 104	1037 ± 56	1216 ± 114	1119 ± 108
Na, mg/100 g DM	1.72 ± 0.40	22.5 ± 5.2	2.25 ± 0.52	7.83 ± 1.80	1.88 ± 0.36	11.4 ± 3.5
Ca, mg/100 g DM	989 ± 237	281 ± 67	856 ± 205	332 ± 80	917 ± 162	306 ± 46
Cd **, mg/kg DM	0.011 ± 0.003	0.027 ± 0.006	0.011 ± 0.003	0.011 ± 0.002	0.014 ± 0.002	0.018 ± 0.004
Hg **, mg/kg DM	0.0069 ± 0.0012	0.0031 ± 0.0006	0.0067 ± 0.0012	0.0022 ± 0.0004	0.0058 ± 0.0004	0.0028 ± 0.0005
Pb **, mg/kg DM	0.086 ± 0.0022	0.086 ± 0.0022	0.10 ± 0.030	0.037 ± 0.010	0.0042 ± 0.0021	0.073 ± 0.0016

** Does not exceed the permitted maximum: Cd—1 mg/kg DM; Hg—2 mg/kg DM; Pb—10 mg/kg DM (the values in Regulation No. 1275/2013). * The results are shown as mean ± CI at $\alpha = 0.05$.

Determination of the content of heavy metals in the feed is necessary since heavy metals have toxic effects on animal health. The analysis showed that heavy metal content did not exceed the permissible norms mentioned in the Commission Regulation (EU) No. 1275/2013 [68] (Table 1).

It was shown that SBT biomass contains fat-soluble and water-soluble vitamins. Vitamin C content was much higher in the stems than it was in the roots and leaves. The leaves of all three SBT cultivars are richer in vitamins E and C. The A vitamin was not found in the SBT stems and roots (n.f.) (Table 2).

Table 2. Contents of vitamins in SBT biomass.

Samples *	Vitamin C, mg/100 g DM‘	Vitamin E (α-tocopherol), mg/100 g DM	Vitamin A (Retinol), mg/100 g DM
MB/LV	15.6 ± 4.4	30.9 ± 4.3	1.29 ± 0.02
MB/ST	178.0 ± 50.0	17.3 ± 2.4	n.f.
BL/LV	12.0 ± 3.0	44.3 ± 6.2	1.14 ± 0.03
BL/ST	9.2 ± 3.6	14.7 ± 2.1	n.f
TAT/LV	13.3 ± 3.6	42.6 ± 2.2	0.86 ± 0.07
TAT/ST	8.0 ± 3.0	16.2 ± 2.6	n.f.
TM/R	n.d.	n.d.	n.f.
OT/R	n.d.	n.d.	n.f.

TAT—Tatiana; BL—Botanicheskaya Lubitelskaya; MB—Maria Bruvele; OT—Otto; TM—Tarmo; ST—stem; LV—leaves; R—roots. * The results are shown as mean ± CI at $\alpha = 0.05$.

Moreover, it has been reported that SBT leaves contain thirteen different amino acids, and wood and bark contain seventeen amino acids [69].

3.6. Main Compounds in SBT Biomass and Their Role in Rumen Digestion

In a complete diet, the amount of crude fiber, protein, and crude fat is of great importance. The total protein content in the dry SBT biomass samples ranged from 18% to 24%/DM (Figure 6).

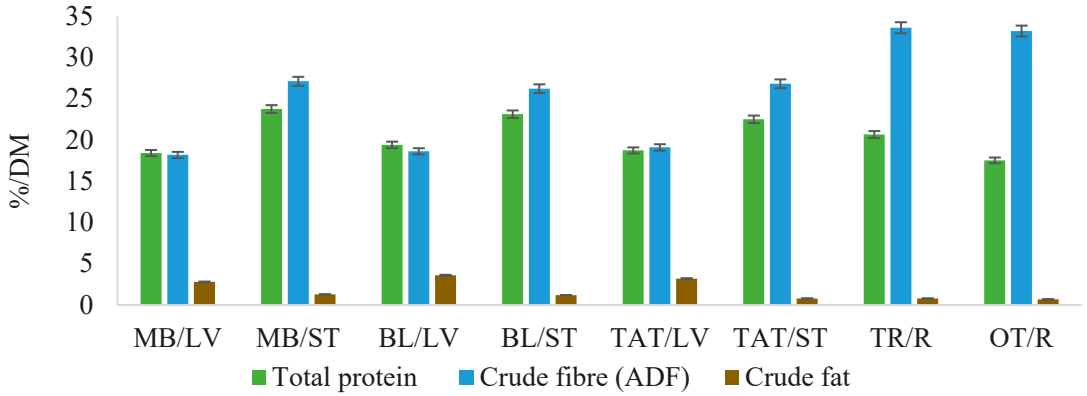

Figure 6. Percentage of total protein, crude fiber, and crude fat in dry SBT biomass (TAT—Tatiana; BL—Botanicheskaya Lubitelskaya; MB—Maria Bruvele; OT—Otto; TM—Tarmo; ST—stem; LV—leaves; R—roots).

The highest content of total protein was in SBT stems ~23%/DM, followed by leaves (18.4–19.4%/DM) and roots (17.5–20.7%/DM).

The content of total fat in SBT biomass was as follows: stems (0.7–1.2%/DM), leaves (2.8–3.6%/DM), and roots (0.7–0.8%/DM). Dairy cows and sheep usually have a pasture-based diet with a low fat content of 2–6% on a DM basis. However, the energy content in fat is more than twice that of carbohydrates, calculated based on weight. Dietary fat contents over 8% can negatively impact rumen function, fiber digestion, and milk production [70]. Thus, it can be said that the fat content in SBT stems and leaves is optimal for nutritional feed.

The crude fiber content in the biomass was 18–27%/DM. Crude fiber is usually indigestible or barely digestible, but it stimulates the production of important gut bacteria. With a deficiency of crude fiber in the diets of cows, an upset of pre-gastric digestion occurs, and the productivity of milk production deteriorates [71]. The highest content of crude fiber in biomass was in SBT roots (33.2–33.6%/DM), followed by stems (26.2–27.1%/DM) and leaves

(18.1–19.1%/DM). Since the crude fiber concentration of SBT roots was ~2 times higher than in leaves, roots could be used for the production of fiber-containing feed additives.

3.7. Determination of the in vitro Gas Production and Digestibility of SBT Biomass

For the evaluation of the feed, methods of measuring digestion by in vitro techniques are ethically preferable, less expensive, and faster than in vivo methods [72]. In the in vitro released gas measurements, the amount of gas that arises from the fermentation process is measured. The high potential of a feed's nutritional and biological value is realized through proper digestion.

The testing was performed on samples from the MB cultivar because it shows better overall composition of the main nutritive compounds. According to in vitro test data, the extract showed the greatest digestibility after the separation of CT. The leaves have a much higher digestibility than the stems. The stems after mechanochemical treatment had a 2.2 times greater digestibility in comparison to the stems before treatment (Table 3).

Table 3. In vitro true digestibility (IVTD) of SBT biomass samples.

Sample *	GP24, mL/g DM	GP48, mL/g DM	IVTD, %/DM
MB/LV	59.97 ± 1.94	71.76 ± 1.61	82.60 ± 4.80
MB/ST/MT	72.38 ± 3.46	83.18 ± 2.21	39.12 ± 6.06
MB/ST	53.99 ± 8.19	65.33 ± 5.56	18.11 ± 4.61
MB/LV: MB/ST/MT (*w*/*w*; 1:1)	76.29 ± 5.73	84.65 ± 7.18	58.11 ± 5.05
MB/LV: MB/ST (*w*/*w*; 1:1)	76.73 ± 5.51	81.93 ± 8.97	49.83 ± 5.16
MB residual fraction after CT separation	134.58 ± 3.94	141.51 ± 4.10	98.69 ± 4.44

ST—stems after CT separation; LV—leaves; R—roots; MB/ST/MT—mechanochemically treated Maria Bruvele stems after CT separation; GP24—gas pressure of 24 h incubated DM; GP48—gas pressure of 24 h incubated DM; IVTD—in vitro true digestibility of feed. * The results are shown as mean ±CI at $\alpha = 0.05$.

It can be seen that the digestibility of the leaves is a bit lower than that of the MB residual fraction after CT separation, but at the same time, the gas emissions are much lower for the leaves. Therefore, for a reduction in GHG emissions, the SBT leaves should always be in the composition of the SBT-based animal feed. The leaves can be combined with other types of biomasses.

The samples from the MB/LV (IVTD = 82.60%) and MB residual fraction after CT separation (98.69%) showed higher digestibility than canola (64.15%), mustard (73.54%), and turnip hays (61.2%), obtained under similar conditions [54], as well as *Quercus robur* L. oak tree leaves (56.22%), alfalfa hay (71.60), giant fennel hey (70.47%) [73], corn silage (61.95%), perennial ryegrass (71.67%), and common vetch/oat hay (66.04%) [74].

Under similar conditions to those in our experiments, the GP24 and GP48 in traditionally used cereal grain forages were as follows: barley—289.5 mL/g DM and 405.8 mL/g DM; wheat—339 mL/g DM and 448.1 mL/g DM; and maize—421.3 mL/g DM and 491.5 mL/g DM, for 24 h and 48 h, respectively. Meanwhile, in the SBT-based samples, the GP for both 24 and 48 h of incubation was several times lower, with only the MB residual fraction after CT separation showing a slightly higher production of gasses (see Table 3); however, even in that case, the gas production was at least two times lower than that of the cereal grain forages [52].

The in vitro testing showed promising results with regard to the future use of SBT biomass as animal feed since all samples had low GHG production accompanied by high digestibility in the leaves and biomass residual fraction after CT separation. Future in vivo experiments will be needed to prove the possibility of the sustainable use of these biomass products as a substitute for some of the traditionally used plant feeds. Moreover, the use of plant biomass with a lower GP will reduce the negative CO_2 imprint from livestock breeding and, thus, will have a positive ecological effect.

3.8. Caloric Value of SBT Biomass

The contents of carbohydrates, lipids, and proteins are stoichiometrically connected with the contents of carbon, hydrogen, and nitrogen. Therefore, based on the results of CHN elemental analyses of biomass, it is possible to calculate a caloric value [75]. The carbon content in the SBT biomass samples varied from 40.5% to 50.9% (Table 4).

Table 4. Elemental analysis and caloric value data of SBT biomass.

SBT Biomass	C	H	N	Organic Matter	Calorific Value, MJ/kg DM		Caloric Value, kcal/g DM	
					HCV	LCV	HCV	LCV
	%/DM; CI ≤ 0.2% at α = 0.05				CI ≤ 0.03% at α = 0.05			
MB/ST	50.6	5.6	3.8	97.2	20.47	19.34	4.89	4.62
BL/ST	49.9	5.6	3.7	98.1	20.21	19.07	4.83	4.55
TAT/ST	50.9	5.5	3.6	97.6	20.52	19.41	4.90	4.64
MB/LV	49.1	5.9	2.7	94.8	19.79	18.67	4.73	4.46
BL/LV	49.3	5.8	3.1	96.2	19.90	18.80	4.75	4.49
TAT/LV	49.5	5.9	3.0	94.8	20.02	18.89	4.78	4.51
TM/R	46.5	4.8	3.3	95.5	18.63	17.51	4.45	4.18
OT/R	40.5	5.0	2.8	96.0	16.60	15.48	3.96	3.70

TAT—Tatiana; BL—Botanicheskaya Lubitelskaya; MB—Maria Bruvele; OT—Otto; TM—Tarmo; ST—stem; LV—leaves; R—roots.

The calorific values of the plant samples were all in the range of 16.6 MJ/kg to 20.52 MJ/kg. The data confirmed that the higher the carbon content, the higher the caloric value, for all the SBT samples. Considering that 1 MJ is 238.85 kcal, the caloric value of the studied biomass was in the range of 4–5 kcal/g DM. The relationship between the carbon content and caloric value with a correlation coefficient of 0.99 is shown in Figure 7.

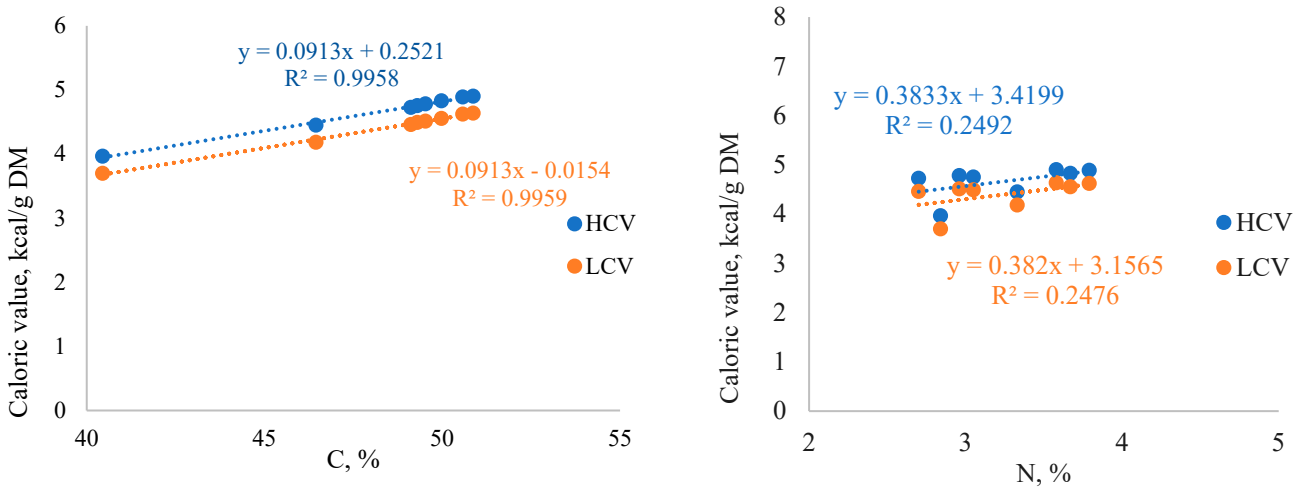

Figure 7. Correlations between C (on the (**left**)) and N (on the (**right**)) element content and caloric value (HCV and LCV).

It can be seen that the N content in the biomass samples does not correlate well with the caloric values of the feed.

3.9. Granulation of SBT Biomass Samples

The granulation of biomass is an effective method for preserving stable quality indicators during the storage of feed and for the improvement of technological characteristics.

MB stems after MT and the separation of CTs and leaves were granulated as described in Section 2.9. Granulation with the roots added (5% of total biomass, correspondingly) was tested to improve the quality of the granules (Figure 8).

Figure 8. Feed pellets: (**A**)—MB/LV: MB/ST/MT (1:1, *w*/*w*) + 5% TM/R; (**B**)—MB/LV; (**C**)—MB/ST/MT.

The characteristics of the obtained pellets are shown in Table 5. In the presence of roots, the biomass became stickier, the durability of the pellets showed a tendency to improve (although insignificantly), and the amount of fines in the pellets was diminished. The roots were also provided to intercalate a sweet taste to the feed. The disintegration of the granules in water was evaluated visually. The time of swelling for the granules made of 100% stems or leaves was 30–60 min.

Table 5. The characteristics of feed granules obtained on the basis of SBT biomass.

Samples	Pellets Durability, %	Pellets Moisture, %	Bulk Density, kg/m^3	Average Length, mm
MB/ST/MT	96.9	5.8	714.8	12
MB/LV	97.7	5.4	715.7	12
MB/LV: MB/ST/MT (1:1, *w*/*w*)	97.2	5.5	714.2	12
MB/LV: MB/ST/MT (1:1, *w*/*w*) + 5% TM/R	98.1	5.6	714.8	8

MB—Maria Bruvele; TM—Tarmo; ST—stem; LV—leaves; R—roots.

The twigs of BL, MB, and TAT, after CT separation, were granulated to obtain fuel pellets. The HHVs of the pellets were 19.8–20.5 MJ/kg (LHV: 18.5–19.6 MJ/kg); durability: 96.8–97.2%; bulk density: 713–715 kg/m^3; ash content: 3.5–3.9%; and the average length of pellets: 12 mm. According to the specifications of the EN ISO 17225 standard [76], the twigs after the separation of CTs can be used for the production of granulated fuel for district heating and power stations. Adding some amount of sawdust would help to diminish the ash content to the level of less than 2%, as required for pellets for non-industrial applications (ISO 17225-2 standard, class B). This study confirmed that the residue of twigs after the isolation of CTs can be used as a granular fuel.

4. Conclusions

This study confirmed that SBT agro-waste biomass, after the separation of condensed tannins, could be a unique and valuable raw material for ruminant feed and feed additive production. Agro-waste biomass, as a side-product of the SBT berry industry, demands no additional agricultural land for the production of the animal feed that supports sustainable agriculture. The high amount of protein, wood fiber, macronutrients, and vitamins in SBT plant material can provide livestock with alternative feed options when other sources of feed are limited. Feed on an SBT basis can also be valuable for animals during winter, and dry seasons, or serve as a supplement to low-protein forage. The anti-microbial properties of the residual fraction after CT separation are useful for particular animal health conditions and for

safe feed storage. The twig fraction, after the separation of CT, is suitable for the production of pellets for district heating and power stations. SBT biomass utilization for animal feed additives and solid fuel allows the creation of a scheme for sustainable SBT berry production, where each target product residual fraction has an added-value application.

Author Contributions: Conceptualization, A.A., S.J. and G.T.; methodology, A.A., S.J., L.L., M.C. and G.T.; validation, A.A., S.J. and M.C.; formal analysis, A.A., S.J., L.L. and M.C.; investigation, A.A., S.J., L.L., N.Z., V.J., L.J., G.R. and G.T.; resources, S.J., G.R. and G.T.; data curation, S.J.; writing—original draft preparation, A.A., S.J. and G.T.; writing—review and editing, A.A., S.J., L.L., N.Z., M.C., G.R. and G.T.; supervision, S.J. and G.T.; project administration, A.A. and G.R.; funding acquisition, A.A., S.J., G.R. and G.T.; All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the ERDF project no. 1.1.1.1/19/A/146 "Biorefinery processing of sea buckthorn non-fruit biomass using innovative techniques and comprehensive analytical investigation, for obtaining prospective high value-added products for the Latvian bioeconomy, including serotonin".

Institutional Review Board Statement: Ethical review and approval were waived for this study since the rumen fluid was obtained from slaughterhouse rams to avoid interfering with animal welfare.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding authors.

Acknowledgments: The authors acknowledge Vizma Nikolajeva for performing the anti-microbial activity analysis, and Anrijs Verovkins for performing the elemental analysis.

Conflicts of Interest: The authors declare that they have no conflict of interest. The funders had no role in planning the study; in the obtainment, analyses, or interpretation of data; in the preparation and writing of the manuscript; or in the decision to publish the results. Ekokompozit Ltd. as a scientific partner in the ERDF project no. 1.1.1.1/19/A/146 declares no conflicts of interest since the ERDF project is a "No-business cooperation project".

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