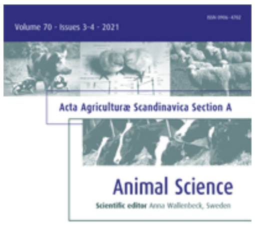

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Juice from silage in green bio refineries – a potential feed ingredient in liquid diets to weaned pigs

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ABSTRACT
The objective of this study was to evaluate silage juice from green bio refineries in liquid diets to pigs and its' effect on growth, cleanliness and health. Ninety-six (L × Y) × H organically raised pigs were fed either a control diet (C-diet) or a silage feed juice diet (SFJ-diet). The C-diet consisted of a commercial feed mixed with water prior to feeding. The SFJ-diet consisted of a lower ration of commercial feed mixed with silage feed juice (SFJ) instead of water, theoretically replacing 10% of the dietary crude protein content. All pigs consumed the juice and grew similarly, on average 0.48 kg/day. SFJ pigs were significantly dirtier on their back and head than C pigs ($P < 0.001$ for all), but cleanliness in the rectum area and in the pen did not differ. Silage juice had only minor effects on hygienic measures and could be a potential local feed ingredient to pigs.

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Bio-refinery; silage juice; growing pig; liquid feed; growth; cleanliness

Introduction

There is a great interest of using small-scale bio-refineries in agriculture as a circular strategy to convert freshly harvested or conserved grass and legumes into valuable and sustainable energy, fuels and feed ingredients to several animal species at farm level (Xiu & Shahbazi, 2015; Hermansen et al., 2017; Franco et al., 2018). Permanent grasslands with clover and other legumes are grown on 44% of the Swedish arable land (Swedish Board of Agriculture, 2021) and in EU grasslands accounts for one-third (31.2%) of the utilized agricultural area (Eurostat, 2020) to provide fodder and forage for livestock. In organic production systems, where the use of artificial fertilizers is banned, mixed leys are essential as the system relies on the legumes for nitrogen supply.

Even though the proportion of arable land with cut ley crop is lower on organic farms without ruminants, it is still grown, as the EU-regulations require roughage to all organic animals. Moreover, the ban on the use of synthetic amino acids in organic feed rations and limited access to high-quality organic protein ingredients has made it necessary to find alternative protein sources for organic pigs and poultry. A key challenge is to find viable, sustainable feed sources from locally produced organic feeds that are tolerable to climate change, which also meets the required levels of nutrients. Ley crops such as grass and legumes can potentially substitute other protein sources for pigs as they have a favourable protein and amino acid composition.

The importance of perennial grass, clover and legumes in crop rotation has in recent years been emphasized in agricultural production due to their favourable effects compared to annual crops. Some examples of these effects are their ability to improve soil fertility, enrich biodiversity and produce high amounts of biomass per hectare with low environmental impacts (Cadoux et al., 2010; Manevski et al., 2018). With regard to the global need to find alternative sustainable protein sources in livestock production, co-production of protein with high biomass productive crops e.g. perennial grass and legumes (Manevski et al., 2017; Solati et al., 2017) in a bio-refinery system could contribute to a more efficient land use (Dale, 2008) and sustain feed and food production.

Protein from green bio-refinery system have been emphasized in recent years and research shows that residual fractions achieved in the bio-refinery processes have good potential as local feed materials. The process includes fractioning of grass and legumes by screw press technique into a solid and a liquid fraction. The liquid fraction, the feed juice, contains nutrients that have a

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higher availability and are better suited for pigs. The main components of the dry matter (DM) are crude protein (CP), minerals, fermentation end products and water-soluble carbohydrates i.e. if residual sugars are present in the silage. From the juice fraction proteins can be further separated into a wet paste by heating and centrifugation, and with further drying, a dry protein concentrate can be recovered. The dried protein concentrate has good potential to replace e.g. soy protein (Stødkilde et al., 2018, 2019; Ravindran et al., 2021). However, the feed juice is a very interesting alternative as feed ingredients for pigs as it can be included directly as a valuable feed component in liquid feeding systems without additional costs for the drying process. The CP content in the juice varies between 166 and 290 g kg^{-1} DM depending on which crops are used as raw material (Franco et al., 2018). Using fresh harvest of grass and legumes limits the possibilities to use it during winter seasons, but harvested and stored as silage, it can be used as a local all-year-round feed ingredient in pig diets. The feed value for the juice fractions that can be extracted from silage is not yet well studied, but according to Keto et al. (2021) the CP and lysine content in silage juice from a first cut harvest of mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) sward was 279 g/kg and 13.6 g kg^{-1} DM, respectively. Research also shows promising results in terms of palatability when silage juice was mixed with cereal-based feed and fed to pigs (Rinne et al., 2018) and on performance, meat quality and gut health of slaughter pigs (Keto et al., 2021).

Co-operation and co-location between bio-refinery and biogas plants can enhance the profitability of biogas plants, thus, also increase the need for grass crops as a raw material. The refining can also enable profitable production of bioenergy at farm level and in collaboration with other actors for the production of protein feed for e.g. pigs. Provided that the nutritive quality of feed juice from either fresh or ensiled grass in a bio-refinery system meets the pig's nutrient requirements, it could be an interesting model for a large proportion of Swedish pig producers as liquid feeding systems dominate the market.

The objective of the present study was to evaluate the applicability to use the silage feed juice (SFJ) from ley crop silage included in liquid diets to weaning pigs. The specific aims were to study the effects of the feed juice inclusion on pig growth, cleanliness and health.

Materials and methods

The study was performed at the pig facility at the agriculture school Sötåsen, Töreboda, Sweden, from October 2020 to April 2021. The study was approved by the Gothenburg Ethics Committee on Animal Research (ethics approval number Dnr 5.8.18-09145/2019), which is in compliance with EC Directive 86/609/EEC on animal studies.

Animals and housing

In total, 96 pigs ((Landrace × Yorkshire) × Hampshire) from four production batches in a certified organic pig production system (KRAV, 2021) were included in the study. The study started at weaning, which occurred at six weeks of age, and the pigs in each batch were then regrouped into four groups in separate pens (six pigs/pen), balanced regarding birth litter and sex. Before the start of the study, all piglets were housed together with their sows in loose housing multi-family pens prior to weaning (from 2 to 3 weeks of age). All pigs were individually marked with an ear-tag at 2–3 weeks of age and were weighed at the start and at the end of the study. Male pigs were surgically castrated under local anaesthesia and analgesic before 5 days of age and all pigs were provided with iron paste before 2 days of age. The age of the pigs was on average 6.2 weeks (±0.26) with a weight of 13.8 kg (±2.84) at the start of the study and the study ended when the pigs were ready for delivery to the finishing unit at 10.9 weeks (±0.46) and an average weight of 29.7 kg (±5.77).

During the study, the pigs (6 pigs/pen) were housed in loose housing pens on concrete floor that were supplied with a thick layer of chopped straw. The pen area was 11 m^2 indoors and the pigs in each pen had access to an outdoor concrete area of 25 m^2. Each pen had a feeding trough for liquid feed (>2 m length) indoors and ad libitum water from a water cup. The pigs had daily access to straw from grass seed production as roughage supply, which was supplied on the outdoor area floor.

Diets and feeding

At the start of the study, the pigs were allocated to one out of two dietary treatments, either a control diet (C-diet) or an experimental diet with SFJ-diet. Each production batch included two pig groups receiving the C-diet and two pig groups receiving the SFJ-diet, thus, in total eight replicates per dietary treatment were included in the study. The C-diet consisted of a commercial feed for weaner pigs, (Smågris SLU KRAV PK, Svenska Foder AB, Lidköping, Sweden, Table 1), mixed with water prior to feeding. The SFJ-diet consisted of a lower ration of the same commercial feed as in the C-diet but mixed with SFJ instead of water. The amount of SFJ was calculated to theoretically replace 10% of the dietary CP content.

Table 1. Ingredient composition of the commercial feed.

Ingredient	% feed
Wheat, KRAV	56.6
Roasted soy bean, KRAV	14.5
Barley, KRAV	10.0
Oats, KRAV	10.0
Hydrolysate from fish protein	3.0
Potato protein	2.0
Mono calcium phosphate	1.3
Calcium carbonate	1.0
Sodium chloride	0.4
Premix	1.2

The SFJ originated from a grass ley with a mixture of timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*), English ryegrass (*Lolium perenne*), red clover (*Trifolium pratense*) and white clover (*Trifolium repens*). The green crop was pre-dried on the field to approximately 25–30% DM and was cut with a forage harvester and chopped to a 20–30 mm particle size. A silage additive with formic and propionic acid was added to the chopped fresh matter. The green crop was ensiled in a bunker silo and stored for at least two months before the opening of the silo. The silage was then included in a bio-refinery system at Sötåsen and comprised of fractioning of the silage by screw press technique into a liquid and solid fraction. The SFJ was collected continuously in buckets and stored in a cooler (for a maximum of 5 days) at 4°C until feeding. At feeding, SFJ or water was mixed manually with the commercial feed and fed in the feeding troughs of the pens. The same amount of liquid was added to both the C-diet and SFJ-diet. The planned feed rations per day over the eight-week study period are given in Table 2. All pigs were fed twice a day and the feed rations were based on the average pen body weight (Andersson et al. 1997).

Registrations and calculations

Health status of the pigs was monitored by daily inspections during the study by the staff and according to the common practice in the herd. Feed, water and SFJ intake were registered by the daily feed rations to each pen, and collection of eventual feed leftovers. The feed and energy conversion ratio were in both SFJ-diet and C-diet based on the intake of commercial feed (kg feed/kg growth or MJ net energy/kg growth).

Table 2. Planned feed ration of the C-diet and the SFJ-diet (kg feed, water or SFJ per pen and day) over the eight-week study period. The pigs were fed twice a day and each pen housed six pigs.

Week	C-diet		SFJ-diet	
	Water	Commercial feed	SFJ	Commercial feed
1	7.6	4.6	7.6	4.2
2	9.0	5.4	9.0	5.0
3	10.8	6.6	10.8	5.8
4	12.4	7.6	12.4	6.8
5	14.4	8.8	14.4	8.0
6	17.0	10.4	17.0	9.2
7	17.0	10.4	17.0	9.2
8	17.0	10.4	17.0	9.2

The cleanliness of the pens as well as the cleanliness and clinical health indicators in individual pigs were registered on day 27 or 28 of the study according to standardized protocols. Pen cleanliness was scored in three sections of the pen (near the feed trough, in the lying area and near the water source) according to a two-grade scale: ok (a maximum of 30% of the surface wet and dirty) or not ok (more than 30% of the surface wet or dirty). The cleanliness of each pig was measured on the back, head and around the rectum area. Cleanliness on the back and head were scored according to a three-grade scale (Score 0: <20% of the skin dirty, Score 1: <50% and >20% of the skin dirty; Score 2: >50% of the skin dirty). Rectum cleanliness was scored according to another three-grade scale (Score 0: clean; Score 1: dirty around rectum but not tail or legs; Score 2: dirty around rectum, tail and legs). Health deviations were scored on individual pigs with regard to body condition, leg health (lameness), shaking, gasping, breathing or sneezing according to standardized two-grade scale (ok or not ok).

Chemical analysis and nutrient declarations

The nutrient value of the roughage was low (not presented) and was assumed not to contribute to the

Table 3. Chemical composition of the commercial feed[#], silage feed juice* and the original silage*. Dry matter (DM, %), net and metabolizable energy (MJ kg^{-1} DM) and nutrients (g kg^{-1} DM) of CP, fat, crude fibre, NDF, ash, lysine, methionine, calcium, phosphorous, sodium and potassium.

	Commercial feed	SFJ	Original silage
DM, %	88.3	10.8	32.0
Net energy	11.2	–	–
Metabolizable energy[¤]	–	10.0	11.4
Crude protein	188.0	157.4	127.0
Fat	61.2	37.0[a]	32.0
Crude fibre	44.2	–	217.0
Neutral detergent fibre (NDF)	–	138.9[a]	409.0
Ash	69.1	120.4	71.0
Lysine	9.8	6.9	–
Methionine	3.3	2.4	–
Threonine	–	5.6	–
Calcium	7.9	7.3	6.4
Phosphorous	7.9	9.5[a]	2.7
Sodium	2.3	1.0	0.6
Potassium	–	36.5	23.1

[#]According to the nutrient declaration of the commercial feed.
*Analysed values according to the laboratory standard methods.
[¤]Metabolizable energy for pigs based on the digestibility coefficient for grass meal.
[a]The analysed values were below the limit of detection, which are presented in the table.

pigs' nutrient uptake to any great extent. Samples of the original silage and liquid SFJ were collected several times during the first pressing occasion, pooled and sent to the accredited laboratory Eurofins (Eurofins Agro Testing Sweden AB, Kristianstad, Sweden) for chemical analysis according to international approved standard methods. Metabolizable energy for pigs was calculated with the digestibility coefficient for grass meal. The chemical composition of the commercial feed is based on the nutrient declaration of the feed. Table 3 presents the chemical composition of DM, energy, CP, crude fat, neutral detergent fibre (NDF), ash, lysine, methionine, calcium, phosphor, sodium and potassium of the commercial feed, the SFJ and the original silage.

Statistical analysis

Statistical analyses were performed using SAS® 9.4 (SAS Institute Inc., 2021). Descriptive statistics were estimated using Procedure Means and Procedure Freq. Pig group was the statistical unit. Differences between treatments in growth, feed conversion in kg and MJ per kg growth, based on the commercial feed in the diets, were analysed with a general linear model using Procedure GLM including the fixed effects of treatment (SFJ-diet and C-diet), batch (1, 2, 3, 4) and the interaction between treatment and batch. Moreover, litter weight at the start of the study was included as a continuous co-variate. Differences between treatments in pen and pig cleanliness (proportion of pigs in the group with each specific score) were analysed with a general linear model using Procedure GLM including the fixed effects of treatment (SFJ-diet and C-diet), batch (1, 2, 3, 4) and the interaction between treatment and batch.

Results

Descriptive statistics (means and standard deviations) of weight, growth and commercial feed, SFJ and water intake are given in Table 4. All pigs consumed the feed, water and juice and grew similarly in the SFJ-diet and the C-diet treatments. The average individual growth of the pigs during the study period was 16.0 and 15.9 kg, thus, on average 0.48 kg/day, respectively. Each pen of the SFJ-diet and the C-diet treatments were provided in total 184 and 205 kg feed, respectively, which were fully consumed by the pigs. There were no significant differences between treatments in average daily growth or in feed conversion ratio (kg feed/kg growth or MJ net energy/kg growth) based on the commercial feed in the diets ($P > 0.05$; Table 5).

According to the daily health checks performed by the staff in the herd, pig health was within normal range in commercial organic production with one exception; one pig in the SFJ-diet treatment in batch 3 died on day 28 of the study. Autopsy showed that the pig had died from ileus, however not considered to be caused by the diet. Health and cleanliness were assessed on day 27 or 28 of the study. No deviations in body condition, leg health (lameness), shaking, gasping, breathing or sneezing were observed. Pigs in the SFJ-diet treatment were significantly dirtier on their back and head than pigs in the C treatment ($P < 0.001$ for all, Table 6), but there were no significant differences regarding dirtiness in the rectum area or cleanliness in the pen ($P > 0.05$ for all, Table 6).

Table 4. Descriptive statistics on individual pig weights, feed, SFJ and water intake (Mean±StD). Individual intake of commercial feed, SFJ and water is based on the amount provided per pen divided with the number of pigs per pen (six pigs/pen). $N = 16$ groups, 8 groups/treatment.

Variable	SFJ-diet	C-diet
Live weight at study start (kg)	14.0 ± 2.04	13.6 ± 2.36
Live weight at study end (kg)	30.0 ± 4.48	29.5 ± 4.62
Days from study start to end	32.9 ± 3.64	33.0 ± 3.70
Commercial feed intake in total (kg)	30.7 ± 5.00	34.2 ± 5.40
SFJ intake in total (kg)	54.3 ± 8.90	0 ± 0.00
Water intake (kg)	1.6 ± 0.40[#]	56.0 ± 8.80

[#]The pigs in the SFJ-diet treatment received 50:50 water and SFJ for the first four days of the study period, in order to acclimatize to the juice.

Discussion

The pigs in the current study showed satisfactory growth with average daily gains (ADG) of 480 g that did not differ between treatments. With the lower amount of commercial feed provided (90% in the SFJ-diet compared to C-diet), we would have expected a 10% lower growth rate if there were no nutrients available for the pigs in the silage juice. To our knowledge, no studies have been performed with SFJ to weaner pigs previously, however the ADG corresponds to the national

Table 5. Effect of treatment on average daily gain, feed conversion ratio (kg feed/kg growth) and energy conversion ratio (MJ net energy/kg growth). LS-means values and standard error (SE). Level of significance $P < 0.05$.

Variable	SFJ-diet	C-diet	SE	*P*-value
Average daily gain from study start to end (kg/day)	0.48	0.49	0.011	0.603
Feed conversion ratio of commercial feed (kg feed/kg growth)	2.05	2.145	0.090	0.497
Energy conversion ratio of commercial feed (MJ net energy/kg growth)	20.32	21.24	0.887	0.497

Table 6. Effect of treatment on cleanliness on pigs and in pens. Results presented as LS-means values and standard error (SE). Level of significance $P < 0.05$. $N = 16$ groups, 8 groups/treatment.

Cleanliness variable	SFJ-diet	C-diet	SE	P-value
Pig (% of pigs)				
Back				
Score 0: <20% dirty	56.3	100.0	3.29	0.001
Score 1: <50 % and >20% dirty	29.2	0.0	4.17	0.001
Score 2: >50% dirty	14.6	0.0	1.47	0.001
Head				
Score 0: <20% dirty	29.2	87.5	2.95	0.001
Score 1: <50% and >20% dirty	43.8	12.5	2.55	0.001
Score 2: >50% dirty	27.1	0.0	2.55	0.001
Rectum				
Score 0: clean	54.2	64.6	10.10	0.487
Score 1: dirty around rectum but not tail or legs	33.3	27.1	9.88	0.667
Score 2: dirty around rectum, tail and legs	12.5	8.3	6.91	0.681
Pen (deviation in cleanliness, % of pens)				
Near feed trough	37.5	12.5	12.50	0.195
Lying area	25.0	12.5	15.31	0.580
Near water source	62.5	62.5	21.65	1.000

average daily gains of 478 g/day reported for weaned pigs in Sweden (WinPig, 2021).

In a study where the diets of growing pigs (11–30 kg live weight) were replaced with either 0, 5, 10 and 15% grass-clover protein concentrate (dried from liquid fraction), the pigs grew on average 16.7, 17.1, 15.9 and 16.8 kg, respectively, and no significant difference was found between pigs with or without grass-clover protein (Stødkilde et al., 2021). This is comparable with the average individual growth of 16.0 and 15.9 kg during the study period for the pigs in the current study. Similarly, Keto et al. (2021) found that fattening pigs fed diets with silage juice extracted from grass-clover silage performed comparable and even better compared to pigs fed a commercial liquid feed, in terms of growth and feed conversion ratio. The amount of silage juice provided by Keto et al (2021) corresponded to a maximum of 3 l per pig (>60 kg live weight) and day. The amount of juice fed in the current study, corresponded to 1.2–2.8 l per pig and day. Even though the energy, CP and lysine content of the silage juice was lower in our study compared with Keto et al. (2021), our results support that silage juice extracted from grass-clover silage can be included successfully in diets to weaner pigs. To our knowledge, data on nutrient digestibility from silage juice in pigs are lacking. In order to estimate the nutritive value of silage juice for pigs, more research on this is needed. The cost-benefit of replacing parts of the commercial feed, in this study around 3.5 kg feed per pig, with SFJ is depending on the market price for the commercial feed in relation to the cost of the production of silage and SFJ. This study only comprised replacement of juice in the pig diet during a 4-week period after weaning and with a moderate level of inclusion. Included in higher levels and during the fattening period would probably improve the cost benefits in the whole production chain. The potential to use juice fractions from green bio-refinery systems has a great potential in livestock feeding and is beneficial both environmentally and economically with improved soil fertility and biodiversity and increased self-sufficiency of e.g. protein feed resources with lower cost for purchased feed (Tidåker et al., 2016).

The pigs fed the SFJ-diet were dirtier on the body and head, however not in the rectum area and there were no differences in the cleanliness of the pens. Due to observations by the farm keepers, the dirtiness was most likely liquid feed residuals, as the pigs fed the SFJ-diet spilled more with the feed around feeding than the pigs fed the C-diet did. Dirtier pigs were also seen among growing pigs fed silage juice in a palatability trial by Rinne et al. (2018). The authors explained this by softened loose faeces, possibly due to a high protein or high potassium content of the silage juice. The content of potassium in the SFJ in the present study was also high. However, as no deviations in cleanliness in the pigs' rectum area or in the pens were observed, only on the back and head, this indicates that the juice per se might have had minor effect on hygienic measures. Moreover, no health remarks were found when the pigs were individually examined on day 27 or 28.

This study was performed at an organic farm under commercial conditions, which might have been a limitation, as the size of the experiment, the feeding and registrations had to be performed manually and with considerations to the capacity of the farm practice and routines. In commercial production systems with liquid feed, feeding routines are more automatized. Still, the results provide important information on the applicability of including silage juice in diets to weaner pigs.

Silage juice can be included in pig diets and provide nutrients and stabilize pH of the liquid feed. It contains soluble components of nutrients and fermentation products such as lactic acid, volatile fatty acids and ethanol from the silage. Consequently, the pH is typically below 4.5, which limits the growth of several potential pathogenic bacteria such as enterobacteria. Weaned pigs have a limited capacity to acidify the gut content that increase the risk of growth of pathogenic bacteria. Fermented liquid feed to growing pigs has been found to decrease the gastric pH, increase the lactic acid in the stomach and reduce the number of enterobacteria throughout the gastrointestinal tract (Canibe and

Jensen, 2003). A diet that can lower the gastric pH may help maintaining intestinal health of the pigs, improve the intestinal morphology (Missotten et al., 2015) and thereby protect the pigs from weaning diarrhoea. In organic pig production, the piglets stay with their sow for a longer time compared with pigs reared in non-organic production systems. This contributes to strengthen the pigs at weaning, still problems with diahorrea occur even though the problem might be of greater concern among pigs that are weaned earlier, at 21–28 days of age. Preliminary results from Nadeau (personal communication, 2021) show that the storage capacity of the press juice can be improved if it is produced from ensiled green pulp, which will have an impact on the hygienic quality of the juice.

It is concluded that perennial grass, clover and legumes in grasslands have a great potential to provide greater biomass and protein production and have a low environmental footprint compared with annual crops such as wheat and maize (Manevski et al., 2018). From an environmental point of view, grasslands are important for increased soil fertility and biodiversity and can contribute to more efficient land use and convert land with poorer conditions into high-quality feed resources. They are important for the increase of carbon storage and reducing the risk of N and P losses in the field, as well as lowering the need for plant protection products compared to growing annual crops (Aronsson et al., 2007; Eriksson et al., 2010). In Sweden, the main part of pig production is located in areas of flat country, where a large proportion of the arable land is based on cereal production, and globally pig diets consist of cereal crops. Long-term monitoring of the agricultural plains in southern Sweden has shown lower soil carbon contents, compared to arable soils in forest districts with large areas of ley crop production. In pig production, the inclusion of grass-derived feeds in pig diets can be a possibility to increase the part of perennial grass into crop rotations and introduce new feed sources, not competing with human resources. However, despite the environmental benefits, research by Tampio et al. (2019) show that there are still challenges with the small-scale bio-refinery system. In order to make optimal use of the green biomasses and use the side streams efficiently as feed or food, improvements in the product quality and bio-refining efficiency are needed. The liquid fraction yield and nutritive quality are dependent on the pressing method and the quality of the fresh raw material and silage.

Conclusions

Press juice from silage can contribute to the nutrient supply to weaner pigs and can be a potential local feed ingredient in liquid feeding systems. Pigs fed the silage juice was slightly dirtier on their heads and bodies, although the cleanliness in their rectum area and the hygiene in the pens were unaffected indicating that the juice had a minor effect on hygienic measures. High content of potassium in the juice calls for awareness. In order to estimate the nutritive value of silage juice for pigs, more studies on nutrient digestibility are needed.

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Disclosure statement

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Author contributions

M. Presto Åkerfeldt: Conceptualization, Data curation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & following; **J. Friman:** Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & following; **F. Dahlström:** Funding acquisition, Investigation, Methodology, Resources, Writing – review & following; **A. Larsen:** Methodology, Resources, Writing – review & following; **A. Wallenbeck:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & following. All authors have read and agreed to the published version of the manuscript.

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