Evaluation of Yucca schidigera plant products for mitigation of gaseous emissions from livestock

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Evaluation of Yucca schidigera plant products for mitigation of gaseous emissions from livestock

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Abstract
Within the 'Regiodeal Foodvalley' project (2020-2024), a literature study was carried out with the aim of giving the project a solid basis for deciding whether or not to investigate additives based on the Yucca schidigera (YS) plant in the ' pilot project' of the project. The literature review shows that YS supplementation is unlikely to substantially reduce ammonia and methane emissions from livestock environments (i.e. more than 20%), although the results of some studies taken together may be indicative of a limited reduction in ammonia emissions. Two broiler studies report no effect of YS supplementation on odor emission in that animal category. Firmer conclusions cannot be drawn from the studies due to their low number, moderate quality and heterogeneity. In the Recommendations chapter, it is recommended to go through a step-by-step R&D process, starting at a relatively simple and relatively cheap laboratory scale in well-defined and controlled conditions, eventually ending in real-life studies. Based on this report, the project decided to continue testing the YS additive on a laboratory scale. Results of these tests will be published in the future.

Synopsis
Binnen het project 'Regiodeal Foodvalley' (2020-2024) is een literatuurstudie uitgevoerd met als doel het project een solide basis te geven om te beslissen over het al dan niet onderzoeken van additieven op basis van de Yucca schidigera (YS) plant in de ‘proeftuin’ van het project. Uit de literatuurstudie blijkt dat het onwaarschijnlijk is dat YS-suppletie de uitstoot van ammoniak en methaan uit veehouderijomgevingen substantieel vermindert (d.w.z. meer dan 20%), hoewel de resultaten van sommige studies tezamen indicatief kunnen zijn voor een beperkte reductie van de ammoniakemissie. Twee studies bij vleeskuikens rapporteren geen effect van YS-suppletie op geuremissie in die diercategorie. Stevigere conclusies kunnen niet worden getrokken uit de studies vanwege hun lage aantal, matige kwaliteit en heterogeniteit. In het hoofdstuk Aanbevelingen wordt aanbevolen om een stapsgewijs R&D proces te doorlopen, te beginnen op relatief eenvoudige en relatief goedkope laboratoriumschaal in goed gedefinieerde en gecontroleerde omstandigheden, uiteindelijk eindigend in real-life studies. Op basis van dit rapport is in het project besloten om door te gaan met het testen van het YS-additief op laboratoriumschaal. De resultaten hiervan worden in de toekomst gepubliceerd.

This report can be downloaded for free at https://doi.org/10.18174/631676 or at www.wur.nl/livestock-research (under Wageningen Livestock Research publications).

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Samenvatting

Introductie
In het project 'Regiodeal Foodvalley' (2020-2024) werkt een breed scala aan organisaties in de zogenaamde Foodvalley-regio in Nederland samen om "de transitie naar een duurzame en gezonde voedselproductie te versnellen". Een van de werkpakketten binnen het project heeft tot doel bij te dragen aan de ontwikkeling en wetenschappelijke beoordeling van emissiearme huisvestingssystemen, emissiebeperkende technieken en voer- en beheersmaatregelen die de uitstoot van luchtverontreinigende stoffen uit stallen kunnen verminderen. Een van de opties die voor het project werden ingediend, was een poedervormig product van de Yucca schidigera (YS)-plant zoals aangeboden door Jadis Additiva B.V. (Schiedam, Nederland). YS-plantenpoeder, toegepast als voer- of strooiseladditief, wordt door de indiener als veelbelovend beschouwd met betrekking tot het vermogen ervan om gasvormige emissies uit stallen te verminderen.

Doel
Het doel van deze studie was om het project te voorzien van een diepgaande en onafhankelijke literatuurstudie over de stand van kennis over het perspectief van YS-additieven (zowel als extract als in poedervorm) om de uitstoot van gassen uit stallen te verminderen. De conclusies en aanbevelingen uit deze literatuurstudie hebben tot doel het project een solide basis te geven om te beslissen over het al dan niet doorgaan met het onderzoeken van deze optie in de 'proeftuin' van het project.

Methodologie
Er is een literatuurstudie uitgevoerd o.b.v. de output van een zoekopdracht via een wetenschappelijke zoekmachine door peer-reviewed tijdschriften, aangevuld met artikelen uit congres proceedings en wetenschappelijke onderzoeksrapporten van universiteiten of onderzoeksinstituten. De indiener was zo vriendelijk zijn literatuurcollectie ter beschikking te stellen van de auteurs, waarin ook werd gezocht naar relevante publicaties. Ongeacht het documenttype is elke publicatie beoordeeld aan de hand van de volgende hoofdvragen:
- is de studie voldoende gedocumenteerd om een solide oordeel over het werk, de resultaten en conclusies mogelijk te maken? Zo ja:
- worden de conclusies voldoende onderbouwd door de verzamelde en geanalyseerde gegevens?
- zijn de gegevens verkregen uit een geldige wetenschappelijke methodologie in termen van onderzoeksopzet, meetstrategie, meetmethoden, gegevensverwerking en statistische analyse?

Resultaten en conclusies
- Op basis van drie geschikte studies naar ammoniakemissie (bij leghennen, vleeskuikens en varkens) en vijf geschikte studies naar methaanemissie (bij melkkoeien, melkschapen, ossen en in vitro) concluderen we dat het onwaarschijnlijk is dat YS suppletie vermindert de uitstoot van die twee gassen aanzienlijk (d.w.z. meer dan 20%) in veehouderijen. De resultaten van enkele studies samen zouden indicatief kunnen zijn voor een kleine reductie van de ammoniakemissie.
- Twee geschikte studies naar geuremissie bij vleeskuikens rapporteren beide geen effect van YS-suppletie op geuremissie in die diercategorie: er zijn meer studies in andere diercategorieën nodig voordat conclusies over geuremissie in het algemeen kunnen worden getrokken.
- Bovenstaande conclusies zijn met de nodige voorzichtigheid getrokken. Stevigere conclusies kunnen niet worden getrokken uit de studies vanwege hun lage aantal, matige kwaliteit en heterogeniteit.

Aanbevelingen
In het hoofdstuk Conclusies worden vijf belangrijke omissies opgesomd die algemeen aanwezig zijn in de bestudeerde literatuur en die het trekken van scherpe conclusies uit die literatuur in de weg staan. In het hoofdstuk Aanbevelingen worden aanbevelingen gedaan om toekomstige studies zo op te zetten dat er robuuste conclusies uit kunnen worden getrokken. Een concrete stap voorwaarts zou kunnen zijn om een stapsgewijze 'onderzoek en ontwikkeling'-benadering te ontwikkelen, beginnend

op relatief eenvoudige en relatief goedkope laboratoriumschaal in goed gedefinieerde en gecontroleerde omstandigheden, en uiteindelijk eindigend in real-life in vivo-studies. Op basis van dit rapport is in het project besloten om door te gaan met het testen van het YS-additief op laboratoriumschaal in goed gedefinieerde en gecontroleerde omstandigheden. De resultaten van deze tests zullen in de toekomst worden gepubliceerd.

Summary

Introduction

In the 'Region Deal Foodvalley' project (2020-2024), a broad array of organisations located in the so-called Foodvalley region in the Netherlands work together to "*accelerate the transition towards a sustainable and healthy food production system*". One of the work packages within the project aims to contribute to the development and scientific assessment of low-emission housing systems, emission mitigating techniques, and feed and management measures that can reduce emissions of airborne pollutants from livestock barns. One of the options submitted to the project was a powderous product from the Yucca schidigera (YS) plant as offered by Jadis Additiva B.V. (Schiedam, the Netherlands). When fed to livestock animals, or added to their bedding, YS plant powder is regarded promising by the applicant with regard to its potential to reduce gaseous emissions from barns.

Objective

The objective of this work was to provide the project with an in-depth and independent literature study on the state of knowledge about the perspective of YS plant products (both as extract and powder) to reduce gaseous emissions from livestock barns. The conclusions and recommendations from this literature study aim to provide the project with a solid basis to make a decision on whether or not to proceed with investigating this option in the 'field lab' of the project.

Methodology

A literature study was performed on the output of a search engine query through peer-reviewed journals, extended with articles from scientific conference proceedings, and scientific research reports produced by universities or research institutes. The applicant kindly made its literature collection available to the authors which was also searched for relevant publications. Regardless of the document type, each study was assessed on the basis of the following main questions:

- is the study sufficiently documented in order to allow a solid judgement of the work, its results and conclusions? If so:
- are the conclusions sufficiently substantiated by the data gathered and analysed?
- are the data obtained from a valid scientific methodology in terms of study design, measurement strategy, measurement methods, data processing and statistical analysis?

Results and conclusions

- On the basis of three suitable studies on ammonia emission (in laying hens, broilers, and pigs), and five suitable studies on methane emission (in dairy cows, dairy ewes, steers and *in vitro*), we conclude that it is unlikely that YS supplementation substantially reduces emissions of those two gases (i.e., beyond 20%) in livestock settings. The results from some of the studies together might be indicative for a small reduction of ammonia emission.
- Two suitable studies on odour emission in broilers both report no effect of YS supplementation on odour emission in that animal category: more studies in other animal categories are needed before conclusions on odour emission in general can be drawn.
- The aforementioned conclusions are drawn with caution. Firmer conclusions cannot be drawn from the studies because of their low number, moderate quality, and heterogeneity.

Recommendations

In the Conclusions chapter, five major omissions are listed that are broadly present in the literature studied and hamper drawing sharp-cut conclusions from that literature. In the Recommendations chapter, recommendations are given to design studies such that robust conclusions can be drawn from those. A concrete way forward could be to develop a stepwise 'research and development' approach, starting relatively simple and relatively inexpensive at lab scale in well-defined and controlled conditions, and eventually ending in real-life *in vivo* trials. On the basis of this report, it has been decided in the project to proceed with testing the YS additive at lab scale in well-defined and controlled conditions. Results from these tests will be published in future.

1 Introduction

1.1 Context

The Netherlands traditionally has a large, knowledge-intensive and innovate agricultural sector. This sector underwent a rapid development mostly in the nineteen fifties to nineteen ninetees. During this period, initially small-scaled and mixed family farms developed along the lines of specialisation, intensification, scaling up, mechanisation, automation, and robotisation. Today, the Dutch agricultural sector is the world's second biggest exporter of agricultural goods with a total value estimated at € 104.7 billion in 2021, of which 72% was Dutch produce and 28% re-export of foreign goods (Jukema et al., 2022). In 2020, the Dutch livestock sector consisted of (WUR, 2022):

- 1.6 million dairy cows at 15,700 farms;
- 476,000 dairy goats at 569 farms;
- 996,000 veal calve places at 1620 farms;
- 32 million laying hen places at 736 farms;
- 49 million broiler places at 637 farms, and;
- 12 million pig places (sows and piglets + fattening pigs) at 3557 farms.

This primary livestock sector is surrounded by an extensive periphery of schools for agricultural education, universities, veterinary practices, animal feed producing companies, companies active in developing housing equipment, feeding systems, ventilation systems, milking systems, air cleaners, and so on. Since the nineteen eighties, the focus on highly efficient and high quality food production has been substantially broadened due to societal and political debate and altered visions on how to produce agricultural goods in a sustainable manner. As a result of EU and national regulations, as well as ambitions formulated by the livestock sector itself, great strides have been made in for example improving animal health and wellbeing, lowering antibiotic use, reducing emissions of airborne pollutants (ammonia, malodorous molecules, greenhouse gasses, dust/bioaerosols), and reducing leakage flows from nutrient cycles (e.g. nitrogen, phosphorus) to soils and waters. At the same time, however, financial margins in the livestock sector are constantly under pressure, partly as a result of cost-increasing effects of this transition against limited possibilities of livestock farmers to pass on their additional price to the food industry and customers. The transition towards a more sustainable food production system has partly been driven by scientific research, innovation and product development, as well as resilience and entrepreneurship of forerunner farmers in response to changing political and societal demands.

1.2 The Region Deal Foodvalley project

In the 'Region Deal Foodvalley' project (2020-2024), a broad array of organisations located in the so-called Foodvalley region[1] in the Netherlands work together to "*accelerate the transition towards a sustainable and healthy food production system*". The Foodvalley region lies roughly between the cities of Utrecht and Arnhem. It is a typical livestock and food production region, characterized by many farms with laying hens and veal calves (to a lesser extent also broilers, pigs, dairy goats, dairy cows), as well as agricultural schools, universities, animal feed producers, food industry, hospitals, et cetera. Region Deals are a type of projects launched by the third cabinet under prime minister Mark Rutte (2017-2022) in which a region in the Netherlands receives co-financing by the national government to make substantial progress on problems and challenges typical for that region. Given the presence of the livestock sector as well as related organisations, the Foodvalley region is

[1] The Foodvalley region is a framework of cooperation involving eight municipalities (Barneveld, Ede, Nijkerk, Rhenen, Renswoude, Scherpenzeel, Veenendaal and Wageningen) with altogether 350,000 residents, and many educational/scientific institutions and businesses related to agriculture and food production.

considered an ideal 'field lab' or 'living lab' to work on the aforementioned transition of the food production system.

The project is divided into three major themes, of which theme 1 focusses on the transition of the primary agricultural sector. Theme 1 can be further divided into sub-projects I through IV and herein: work packages 1 through 12 (Figure 1).

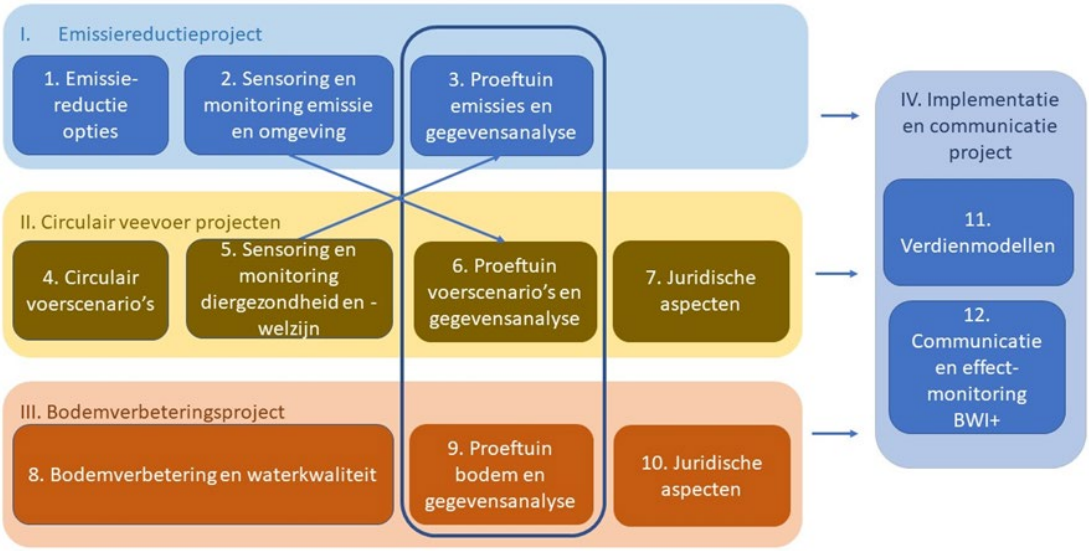

Figure 1 *Structure within theme 1 of the Region Deal Foodvalley project. English translation: I: Emission reduction project, II: Circular feed project, III: Soil improvement project, IV: Implementation and communication project, 1: Emission reduction options, 2: Sensoring and monitoring emissions and environment, 3/6/9: Field labs and data analysis, 4: circular feed scenario's, 5: Sensoring and monitoring animal health and welfare, 7/10: Legal aspects, 8: Soil improvement and water quality, 11: Business models, 12: Communications and effect monitoring.*

Work package (WP) 1, within project I of theme 1, aims to contribute to the development and scientific assessment of low-emission housing systems, emission mitigating techniques, and feed and management measures that can reduce emissions of airborne pollutants from livestock barns. The organisation of WP1 is carried out by the Poultry Expertise Centre (PEC, part of the agricultural school community Aeres Group; Barneveld, the Netherlands) whereas Wageningen Livestock Research (WLR; Wageningen, the Netherlands; part of Wageningen University and Research) performs the scientific work in this WP. The farmers union 'LTO Noord' sets up the field lab with affiliated livestock farms.

The assessment of emission reduction options takes place in the so called 'field lab' (or 'living lab'; 'proeftuin' in Dutch), which is the term for all facilities (commercial farms as well as labs and experimental facilities) within the Foodvalley region, available to the project. As becomes clear from Figure 1, options for emission reduction are assessed from an integral view: options should not only reduce emissions, but also have no side effects, or even have beneficial effects, on aspects like animal health and wellbeing, closing nutrient cycles, and the earning potential of farmers. Furthermore, the focus of WP1 is on the following animal categories within the livestock sector: 1) poultry, 2) veal calves, 3) dairy goats, 4) pigs, and 5) dairy cows.

Companies that have interesting options for emission reduction that are close to market release within the innovation process, are asked to submit their option to the project. Subsequently, PEC carries out intakes with each applicant and produces an extensive dossier for each option. These dossiers are then assessed by an independent Expert Team (ET), which members are active as livestock farmer, livestock consultant, lecturer, environmental scientist, animal feed scientist, or veterinary scientist. Upon discussing the details of the dossiers, the ET issues an advice to the project team on the general perspective of the submitted option. Based on this advice, the project team decides on which options are granted access to the project and its field lab.

1.3 This study on Yucca schidigera plant products

One of the options submitted to WP1 was a powderous product from the Yucca schidigera (YS) plant as offered by Jadis Additiva B.V. (Schiedam, the Netherlands). When fed to livestock animals, or added to their bedding, YS plant powder is regarded promising by the applicant with regard to its potential to reduce gaseous emissions from barns, such as ammonia (NH_3), methane (CH_4), and odorous molecules. After studying the dossier on this option, the Expert Team (ET) judged that the evidence on the perspective of YS was inconclusive and that an advice on whether or not to proceed with this option in the project first required an in-depth and independent literature study. Subsequently, the project team decided to follow this advice. Scientists of Wageningen Livestock Research then carried out this literature study.

1.4 Objective

The objective of this work was to provide the project Region Deal Foodvalley with an in-depth and independent literature study on the state of knowledge about the perspective of YS plant products (both as extract and powder) to reduce gaseous emissions from livestock barns. The conclusions and recommendations from this literature study aim to provide the project with a solid basis to make a decision on whether or not to proceed with investigating this option in the 'field lab' of the project.

1.5 Outline of this report

This report follows the structure of a literature study and advisory report. In chapter two, details are given on the methodology followed in this work. Chapter three presents the results gathered from the literature and discusses those results in the light of the objective. Chapter four presents the conclusions drawn. Chapter five, lastly, provides a number of recommendations. A list of all literature cited is provided as the last element of this report.

2 Methodology

This literature study was carried out in the second half of 2021. The main body of literature was found by consulting the Elsevier Scopus website. Literature searches were done using the search terms: 'yucca schidigera' AND at least one of the terms 'livestock', 'dairy cows', 'pigs', 'poultry', 'ammonia emission', 'methane emission', 'odour emission'. This search strategy resulted in a total of 80 publications (this number included duplicates from the different search terms). The titles and abstracts of the publications were reviewed for their relevance for this literature study, after which 24 peer-reviewed publications were selected. These included 11 review articles and 13 original research articles. The excluded publications did not provide information on the animal categories within the focus of this literature study or did not concern feed management interventions.

Subsequently, a meeting was organised at June 24th, 2021 between two employees working at the applicant and two of the authors of this work. This meeting was aimed at clarifying the aim and outline of the literature study to the applicant, introducing the company and emission reducing option of the applicant to the researchers, and answering questions that arose from both parties.

Upon the meeting, the applicant kindly made their literature collection available for use in this literature study. This collection was also searched for relevant publications. A total of 74 documents were provided by the applicant:

- of which 2 were the company's brochure in Dutch and English (excluded);
- of which 1 publication was only available in German;
- of which 9 documents concerned animals other than livestock, such as rabbits, rats, … (excluded);
- of which 62 documents on effects of YS in livestock;
    - of which 17 documents were not peer-reviewed;
    - of which 33 were peer-reviewed journal publications;
        - of which 1 was an *in vitro* study;
    - of which 11 papers concerned reviews that were used as background information.

The literature studies included peer-reviewed journal articles, articles from presentations at scientific conferences, and scientific research reports produced by universities or research institutes. Regardless of the document type, each study was assessed on the basis of the following main questions:

- is the study sufficiently documented in order to allow a solid judgement of the work, its results and conclusions? If so:
- are the conclusions sufficiently substantiated by the data gathered and analysed?
- are the data obtained from a valid scientific methodology in terms of study design, measurement strategy, measurement methods, data processing and statistical analysis?

3 Results and discussion

3.1 Potential modes of action of Yucca schidigera

3.1.1 Active components: glyco-components and saponins

Active components in Yucca schidigera (YS) products are glyco-components (i.e., sugars attached to another organic molecule) and saponins (i.e., steroid glycoside molecules that show soap-like properties in water). Although literature shows inconsistent results, biological effects of YS are thought to be mainly attributed to the presence of saponins (Abdel-Raheem et al., 2019).

Saponins are naturally produced by certain bacteria and lower marine animals as well as by plants. Because they contain both water-soluble and fat-soluble components, saponins can be regarded as natural detergents (Cheeke, 1999; Chepete et al., 2012). According to a review by Francis et al. (2002), the saponin content of plants can vary; it is affected by physiological age, environmental factors and agronomic factors. In general, saponin content tends to be higher in immature plants of a species, where they can have various functions such as microbial, antifungal, antiviral and anthelminthic activities. In some plants, a stimulating factor for the saponin content may be light availability during germination (Francis et al., 2002). Saponins are found in both wild and cultivated crops. In plants used for their herbal and health-promoting properties, the steroid form of saponins is the most abundant (Addisu & Assefa, 2016). Likewise, in YS, the main active component is the steroid form of saponin (Ayoub et al., 2019; Amber et al., 2004).

3.1.2 Mode of action in ruminants

In ruminants, some studies suggest that feed conversion can be improved by YS supplementation. According to Liu et al. (2021), it is not known which components in YS could enhance nutrient acquisition and utilization. They suggest that saponins present in YS may slow down passage rate which results in improved feed efficiency. In their study using dairy calves, feed-to-gain ratio was determined after YS supplementation (maximum dose of 9 g YS powder/day). However, only a significant quadratic dose effect was found, with the lowest feed-to-gain ratio at 6 g YS/day. The linear effect with increasing YS dosages was not significant (Liu et al., 2021). Accordingly, in their lamb study, Kaya et al. (2006) did not find significant effects of 150 ppm YS extract on feed conversion ratio. De Sousa et al. (2019), interestingly, found a statistically significant higher feed efficiency in veal calves with 2g YS extract/day compared to 0 and 1 g YS extract/day.

It should be noted however, that improving the feed efficiency in livestock (i.e., improving the nitrogen absorption in the gastro-intestinal tract and/or improving the nitrogen fixation in growth, eggs or milk) as such does not necessarily lead to reduced ammonia emission from urine (cows, pigs) or feces (poultry). Under practical barn conditions, factors influencing the ammonia generation and volatilisation (e.g., water availability for microbial activity, the amount of soiled surface area, pH, temperature, air velocity) are the key factors, next to the amount of nitrogen excreted.

Given the varying results with regard to feed efficiency, in ruminants, saponins are also suggested to have lytic (destructive) activities on cell membranes. They might cause lesions that are suggested to be similar to micelle aggregation of saponin and cholesterol in cell membranes. Through this mechanism, saponins can block the activity of methanogens (i.e., microorganisms that produce methane as a metabolic by-product) indirectly, by altering cell membrane permeability of rumen protozoa (Abdel-Raheem et al., 2019; Beauchemin et al., 2009). Saponins attach to cholesterol/lipid sterol in the cell membrane of protozoa, causing a curved cell membrane, pore formation or lipid raft disruption. This results in breakdown of the cell, cell lysis, and cell death (Das et al., 2012). The protozoa population in the rumen plays an important role in methane production. Ciliate protozoa provide H_2 as a substrate for methanogens. These protozoa represent 9 to 25% of ruminal

methanogens (Sun et al., 2017). Ruminal cycling of microbial N and efficiency of microbial CP synthesis are largely controlled by ciliate protozoa. Reduction of protozoal activity can thus improve dietary N utilization and increase microbial CP flow to the intestine. When ruminal ammonia concentration is high, YS extract is able to bind ammonia and to release it again when ruminal ammonia concentration is low. This results in a continuous ammonia supply required for microbial protein synthesis (Das et al., 2012).

Five studies were found investigating effects on ruminal protozoa counts. Table 1 presents an overview of the study designs and outcomes.

Table 1 *Overview of studies on effects of YS supplementation on ruminal protozoa count in dairy cows. Percentages in bold indicate a significant effect of YS treatment.*

Dairy cows	Experimental period	Number of animals	Mean BW	Yucca treatment	Diet	Experimental facility	Method	Effects of YS/saponins/sarsaponins on ruminal protozoa count
Abdel-Raheem et al. (2019)	4 months	3 groups of 5 buffalo calves	167 ± 3.5 kg	0; 1 g YS powder/kg DM in concentrate mixture; 2 g YS /kg	Concentrate mixture (2% of BW; 14.75% CP), wheat straw (2,76% CP), Egyptian clover (17,64% CP), roughage level 1% of BW + 3 different dosages of YS	Separate pens with concrete floor and equipped with locally manufactured feed manger	Stomach tube sample filtered through cheesecloth	Total ruminal protozoa count: Control: 4.48 x 10^6 **1g YS/kg DM: -34%** **2g YS/kg DM: -32%**
Holtshausen et al. (2009)	3 28-day periods	2 groups of 6 cows	627 ± 55 kg	0 or whole-plant YS powder at 10 g/kg of DM	Control diet (52,1% DM; 17.0% CP) 51:49 forage:concentrate ratio + 2 different dosages of YS	Individual tie stalls fitted with rubber mattresses and bedded with wood shavings; environmental chambers for methane production measurement during last week of period	Fuchs-Rosenthal counting chamber	Total ruminal protozoa count: Control: 6.15 x 10^5/ml 10g/kg YS: +0.3%^{ns}
Hristov et al. (1999)	14 days of adaptation + 14 days of sample collection	6 heifers	443 ± 6.1 kg	0; 20; 60 g YS powder/day	Alfalfa silage:barley grain-based diet	Not documented	Fuchs-Rosenthal counting chamber	Ruminal protozoa count: Control: 0.69 million/cm^3 **20 g YS: -42%** **60 g YS: -20%**
Steers								
Lila et al. (2005)	3 periods of 14 days of adaptation + 4 days digestion trial	3 Holstein steers	248 ± 27 kg	0; 11.2; 22.4 g sarsaponin per 2.25 kg DM (equal to 0.5% and 1% sarsaponin of DM)	Sudangrass hay + concentrate mixture at a ratio 1.5:1 twice daily; and sarsaponin (0, 0.5 and 1% of DM)	Digestion stalls	Rumen fluid diluted with methylgreen-formalin-saline	Total ruminal protozoa count 5hrs after feeding: Control: 7.3 x 10^5/ml **0.5% sarsaponin: -34%** **1% sarsaponin: -36%**
Goats								
Santoso et al. (2007)	4 periods of 14 days experiment + 8 days of adaptation + 1 day of sampling	4 Kacang goats	20.3 ± 2.78 kg	0; 13; 19.5; 26 mg saponin/kg BW	Elephant grass silage and grain-based concentrate (70:30 on a DM basis)	Individual metabolism cages	0.1mm depth Neubauer counting chamber	Total ruminal protozoa count: Control: 13.6 x 10^4/ml **13 mg/kg BW saponin: -34%** **19.5 mg/kg BW saponin: -40%** **26 mg/kg BW saponin: -41%**

According to Abdel-Raheem et al. (2019), saponins can decrease protozoal activity through interactions with the cholesterol of protozoal cell membranes (table 5). In their 14-day digestibility trial, protozoa count in rumen contents were measured by filtering samples through one layer of cheesecloth. The study showed a significant decrease in the number of total protozoa after treatment with 0, 1 or 2 g YS powder/kg diet. According to Abdel-Raheem et al. (2019), saponins can decrease ruminal protein breakdown and subsequently lower ruminal ammonia-N levels, by regulating the ammonia release in the ruminal digestive tract (Abdel-Raheem et al., 2019). However, the method used is not very accurate, and moreover, most often between 2-4 layers of cheesecloth are used to count protozoa numbers.

In the in vivo part of the study by Holtshausen et al. (2009), ruminal protozoa numbers were counted in 12 cows. Numbers were measured at 5 time points during each 28-day period using a Fuchs-Rosenthal counting chamber. Results show that protozoa count was not affected by YS supplementation. This was in line with the lack of effects on ammonia N concentration of the ruminal fluid in their study.

Lila et al. (2005) studied the effects of sarsaponin on ruminal protozoa count in steers. Sarsaponin was added to the diet (0; 0.5; 1% of DM) of three Holstein steers for four days. On the final day of each treatment period, 2 and 5 hours after morning feeding, rumen fluid was collected. The rumen fluid was diluted with methylgreen-formalin-saline and analysed for its ciliate protozoa count. In this study, the protozoa count was decreased linearly and significantly by sarsaponin treatment. However, in this study design, only three cows were used. Moreover, as no long-term effects of sarsaponin on

the protozoa count were measured, no conclusions could be drawn on ruminal adaptation to sarsaponin.

In the study by Santoso et al. (2007), four goats were used to study the effects of saponins on protozoa count. On day 14 of each experimental period, rumen fluid was collected to count for protozoa. The protozoa number decreased linearly and significantly with saponin treatment. According to Santoso et al. (2007), the observed effect could be due to the affinity of saponins towards cholesterol, because cholesterol is only present in eukaryotic cell membranes, such as protozoal cell membranes.

In a study by Hristov et al. (1999), 6 heifers were fed 0 (control), 20 or 60 g YS powder (dried and pulverized whole plant) per day. Duration of the experiment was 14 days of adaptation to treatment followed by 14 days of sample collection. Rumen content samples were collected on day 15, 16 and 17 of each period, 0, 2, 4 and 6 hours after YS supplementation. The bottom of the ventral sac and the reticulum were combined and squeezed through two-layered cheesecloth. The filtrate was used to count protozoa numbers. Protozoa concentration was significantly reduced by both YS treatments. This concentration decreased most at the dosage of 20 g YS (-42%) and increased again at 60 g YS (-20%). Given these observations, it was concluded that no ruminal microflora adaptation had occurred, because according to the authors, it would have begun during the 14 days before the sampling period. Hristov et al. (1999) would have expected the flow of microbial protein to the intestine to increase as a result of the decreased protozoa count; as increased absorption of amino acids is beneficial for the animal. However, no significant differences in protein flow were observed.

Since protozoa provide hydrogen as a substrate for methanogens, a reduction in protozoa count may lead to a reduction in methanogen population; which may result in a reduction in methane emission. The same mechanism holds for ammonia: protozoa count is reduced by saponins, which reduces bacterial ingestion by protozoa and in turn predation intensity. This may result in a decrease in ammonia concentration. Saponins can also contribute to an interaction between ammonia and sugar moiety of substances which reduces ammonia availability (Jayanegara et al., 2014).

In their review, Francis et al. (2002) conclude that saponins act non-specific on protozoa. Their toxic property may be a result of the detergent effects on cell membranes and could be reduced upon deglycosylation (Francis et al., 2002). From their review, Beauchemin et al. (2009) concluded that *in vitro* saponin addition can reduce protozoal activity and methane production. However, the same effects are not always observed *in vivo*. This could be the result of the use of different saponin sources and different saponin dosages. Moreover, the latter is inconsequently documented in literature. Adaptation of the rumen microflora to the presence of saponins could also play a role. In other reviews, a lack of effects of YS supplementation on ammonia and methane emissions is also suggested to relate to adaptation of rumen microbial population of the animals (Das et al., 2012; Beauchemin et al., 2009).

3.1.3 Mode of action in monogastric animals

In monogastric animals, some studies suggest that feed conversion can be improved by YS supplementation. Alagawany et al. (2016) reported some statistically significant effects of YS on feed conversion ratio (FCR; kg feed per kg production) in laying hens. Only during 36-40 weeks of age, FCR was statistically significant lower (-23%) for the group that received 150 mg YS extract/kg of basal diet (saponin level not reported). The authors hypothesise this finding to be due to emulsification of oil fats (which enhances fat digestion) and higher absorption of nutrients. However, the effect was not linear. No statistically significant effect was found with increasing age up to 52 weeks. Thus, it seems that the reported difference in FCR at 36-40 weeks of age may well be a coincidental finding, unrelated to YS supplementation.

Sahoo et al. (2021) found a statistically significant lower FCR upon YS extract supplementation (-10%; 125 mg YS/kg feed) in broilers, which was supported by a statistically significant improved protein efficiency ratio. Similarly, Su et al. (2016) studied the effects of YS extract on feed efficiency in

broilers. At a dosage of 100 and 200 mg YS extract/kg, FCR was statistically significant lower from day 14 to 42 (-7%).

In a study by Alagawany et al. (2018), FCR in quails was actually significantly increased by YS extract (100 and 200 mg/kg diet resulted in a +7% and +14% FCR). Ayasan et al. (2005) reported a statistical tendency for a lower FCR at 120 ppm YS powder in quails (-11%, P>0.05). Although not statistically significant, the authors attribute this effect to better absorption and utilisation of nutrients. Likewise, in a broiler study by Cabuk et al. (2004) and a laying hen study by Kutlu et al. (2001), FCR did not differ significantly with YS treatment (0.120 mg YS/kg, 120 mg YS powder/kg diet, respectively).

Overall, two studies in broilers found statistically significant improvements of the FCR of 10% and 7%, and a third study in quails a statical tendency for 11% improvement. On the other hand, one study in broilers and two studies in laying hens found no effects on FCR. Lastly, a seventh study found a worsened (i.e., higher) FCR of 7 and 14%. The general picture from all seven studies in poultry suggest no substantial effect of YS supplementation on FCR.

It should be noted, that improving the feed efficiency in livestock (i.e., improving the nitrogen absorption in the gastro-intestinal tract and/or improving the nitrogen fixation in growth, eggs or milk) as such does not necessarily lead to reduced ammonia emission from urine (cows, pigs) or feces (poultry). Under practical barn conditions, factors influencing the ammonia generation and volatilisation (e.g., water availability for microbial activity, the amount of soiled surface area, pH, temperature, air velocity) are the key factors, next to the amount of nitrogen excreted

3.1.4 Mode of action upon secretion of urine and feces

YS products could furthermore be active after nitrogen compounds have been excreted by livestock. For instance, Panetta et al. (2006) hypothesise that ammonia emission can be reduced by YS supplementation through the binding of excreted N or a reduction in ammonia-N concentration. This was, however, not confirmed by their study. The same hypothesis was put forward by Min et al. (2001), who suggest that ammonia levels could be reduced by YS by binding ammonia in feces of pigs.

Francis et al. (2002) suggest in their review that saponins present in YS extract have the potential to entrap ammonium-N from straw supplemented with urea. By this entrapment, rumen bacteria could be exposed to a higher nutrient availability. This could lead to decreased ammonium losses to the air, through which environmental effects can be reduced (Francis et al. 2002). Similarly, in monogastric animals, YS is suggested to reduce urease activity, which results in suppression of ammonia formation and emission. According to the review by Saeed et al. (2018), YS is able to kill pathogens, inhibit urease, bind ammonia and lower the pH value of poultry litter. However, the substantiation for these statements is unclear.

3.1.5 Magnitude of effects in relation to saponin source and harvesting

Different methods have been described for harvesting of saponins. Generally, the plant material is extracted with aqueous methanol or ethanol. Subsequently, the extract is evaporated under reduced pressure, after which the extract is separated into n-butanol and purified further. To reach a highly pure extract, these steps may have to be repeated. Different analytical methods are suitable to determine the composition of saponin extracts such as mass spectrometry (MS), proton and carbon nuclear magnetic resonance (NMR) and infrared spectroscopy, thin layer chromatography (TLC), and staining with dehydrating reagents and hydrolyzation (Francis et al., 2002). Different extraction methods may lead to a variation in active components of YS extract; meaning that observed effects in studies could not solely be attributed to saponins.

Furthermore, different sources of saponins may also vary in their active components. Even within a single plant species, a large variety of saponin structures can be found (Jayanegare et al., 2014). Plant contents can be influenced by the vegetative stage of the plant at harvest (Sun et al., 2017).

Due to the variety of saponins with similar chemical structures and properties, clear functionality and its relationship with structure is difficult to establish (Francis et al. 2002).

Besides extraction, saponins from Yucca schidigera can be present in products in a powderous form. The applicant to the Region Deal Foodvalley project, Jadis Additiva BV, states that their product is harvested from the stems of the YS plant (not the leaves and branches) and pulverised by a hammer mill to a powderous product. Batches are sampled and analysed for their saponin content, after which batches are mixed to yield a homogenous product with a guaranteed saponin content of >10.5%. Furthermore, no other active compounds are mixed in the powder. According to the applicant, the powderous form of YS has distinct effects from the extract form generally studied in literature due to the higher and standardized amount of active ingredients.

As a result of differences in plant sources, isolation or grinding, and possible additions of other compounds to the product, YS products may vary in the amount of saponins as well as the presence of other active components. In studies, usually the dose of YS extract is reported, rather than the actual dose of saponins within this extract. This hampers comparison of studies investigating the same end points as well as drawing general conclusions on the perspective of YS products in reducing gaseous emissions from livestock.

3.2 Safety and potential side effects

Little literature was found regarding toxicity studies on YS. In a study by Wisløff et al. (2008), YS juice was tested for its nephro- and hepatotoxicity in 30 lambs. The experimental doses of 1.5 g (63 mg sapogenin) and 3.0 g (126 mg sapogenin) YS juice per kg live weight led to a number of toxic effects. Due to acute renal failure, 12 lambs died or had to be euthanised. According to the authors, renal damage by saponins is not usual. They suggest that the membrane-permeabilising ability of saponins may have damaged renal epithelial cells, or that saponins may have facilitated the uptake of other nephrotoxic substances present in YS juice. Saponins were also detected in the liver of the lambs. In 9 lambs that were euthanised at the end of the study, PAS-positive material had accumulated in hepatocytes. Other signs of disease in the lambs were diarrhoea and dehydration.

At present, YS is registered as a feed additive in the European Union as a natural product (botanically defined) (EU 1831/2003). This general registration does not contain further specifications, meaning no specific functional claims can be made. Currently, no risk assessment by the European Food Safety Authority (EFSA) is available; an EFSA risk assessment application was submitted for the use of YS as a feed additive for poultry. At the moment, YS is included in the EU catalogue of feed materials (EU, 2017; product numbers 7.12.1 (powder) and 7.12.2 (juice)). This means that YS powder and juice are allowed to be included in animal diets, but it is not allowed to make claims regarding specific effects of these products, e.g. on the emission of ammonia. For use in food, YS powder (dried and finely powdered logs) and YS extract (pressed and condensed juice) are Generally Recognized As Safe (GRAS) by the U.S. Food and Drug Administration (FDA; 21CFR172.510). The GRAS label allows the use of YS powder and extract in food. The safety of the use of YS extract and YS powder in feed are not yet assessed by the FDA. Yucca schidigera can be found in many (pet) food products.

3.3 Studies on ammonia-related effects

3.3.1 Poultry

Seven studies were found on ammonia-related effects of YS in poultry. Table 2 presents an overview of the study design and outcomes.

Table 2 *Overview of studies on ammonia-related effects of YS supplementation in poultry. Percentages in bold indicate a significant effect of YS treatment.*

	Experimental period	Number of animals	Mean BW	Yucca treatment	Diet	Experimental facility	Method	Ammonia-related effects of YS
Laying hens								
Chepete et al. (2012)	12 weeks	4 groups with 6 replicates with 3 hens per replicate	Unknown	0; 50; 100; 200 ppm YS powder	Standard laying-hen diet + 4 different dosages of YS	24 metabolic cages	Gaseous Emission Vessels System	Ammonia emission rates from manure: Control: not specified All treatments: no significant differences among measurement sessions **100 ppm YS: 370 mg/d per kg of manure; mean difference of -29% when compared with pooled data of all other treatments**
Broilers								
Patoary et al. (2020)	28 days	4 groups with 3 replicates with 20 birds per replicate	43.2 ± 0.3 g	0; 1ml YS extract per 16 liters of drinking water; 1ml YS per 20L; 1ml YS per 24L	45.% corn; 17.0% soybean meal; 14.1% CP + 4 different dosages of YS	Open-sided broiler house	Micro Essential pHydrionTM ammonia meter tester paper with color chart	Ammonia emission from litter: Control: 25.87 **1ml YS/16L: -55%** **1ml YS/20L: -42%** **1ml YS/24L: -23%**
Ayoub et al. (2019)	35 days	3 groups with 6 replicates with 15 birds per replicate	173.55 g	0; 0.5ml YS extract per liter drinking water; 1ml/L	Starter diet (day 0-21); 22.4% CP; 55.6% yellow corn; 36.1% soybean meal. Finisher diet (day 22-35); 18% CP; 68.36% yellow corn; 21.5% soybean meal	Deep litter system	Kjeldahl method	N content of litter: Day 21 control: 0.813% **0.5ml YS/L: -12%** **1ml YS/L: -37%** Day 35 control: 1.20% **0.5ml YS/L: -40%** **1ml YS/L: -55%**
Cohuo-Colli et al. (2017)	50 days	4 groups with 3 replicates with 23 birds per replicate	Unknown	1 kg/m^2 litter density; 1 kg/m^2 litter density + 125 ppm YS (MicroAid); 2 kg/m^2 litter density; 2 kg/m^2 litter density + 125 ppm YS (MicroAid)	Unknown	2 farms with 23 chickens and 3 pens per treatment	Dräger X-am 5000 gas detector	Ammonia emission from litter: Low density litter control: 15.79 ppm **125 ppm YS: -20%** High density litter control: 12.47 ppm **125 ppm YS: -17%**
Cabuk et al. (2004)	20 days	4 groups with 5 replicates with 48 birds per replicate	639.57 g at 21 days; 1911 g at 42 days	0 or 120 mg YS/kg	Isocaloric and isonitrogenous diets	Wood shavings' litter in floor 25 pens with open-sided naturally ventilated broiler house	Dräger gas detector pomp (model 21/31)	Ammonia concentration of the broiler house Control: 21.25 ppm **0.120 g/kg YS: -15%**
Lazarevic et al. (2014)	42 days	902 broilers	44 ± 0.003 g	500 g De-Odorase/t	Corn-based, commercial broiler feed in three phases (starter, grower, and finisher; 86.5% DM, 21; 19; 18% CP)	Rooms with one control pen and one treatment pen	Dräger tubes type 2a	Litter ammonia concentration: Control: 5.47 ppm **500 g De-Odorase/t: -17%**
Raumberg-Gumpenstein (Jadis Additiva B.V. 2019)	29 days	2 groups with 2 replicates with 210 birds per replicate	Mean slaughter weight 2117.4 g	125 g per 1000 kg (saponin content ≥10.5%)	Starter = 22.5% CP; midway feeding phase = 21.5% CP; final fattening phase = 21.5% CP	Two barns with two sections, groups were rotated after each cycle	INNOVA 1412 Multi Gas Monitoring Instrument, LumaSense Technologies	Average ammonia emission in exhaust air: Control: 5.88 ppm **0.125 g YS/kg: -14%**

In a 12-week study by Chepete et al. (2012), 72 laying hens were divided over four treatments, each consisting of six replicates with twelve hens (table 1). The hens were fed diets with 0, 50, 100 or 200 ppm YS powder daily. Because 100 ppm was the recommended level by the industry, this dosage was used as the positive control. Production performance (i.e. feed intake, egg production) was not affected by treatment with YS powder. Manure (slurry) from week 11 and 12 was collected from all treatment groups. The samples were used in a vessel experiment using eight vessels (two samples x four regimens). Subsequently, ammonia emissions from the substrate were measured during three successive sessions of three days using the Kjeldahl method. In these measurements, an air mass flow controller was used to control and measure the flow rate of supplied fresh air. No significant differences were found between treatments; as these calculations were not included in the report, interpretation of the differences between treatments is not possible. Because of the insignificant results, the authors decided to pool the data from the three sessions for each treatment and to compare them with the 100 ppm dosage (industry-recommended level). At a dosage of 100 ppm, ammonia emissions from the vessels were reduced significantly, on the first (-44%) and second (-28%) day. Reductions by the 100 ppm dosage measured from the third day were not significant (-14%). Moreover, ammonia emission for the 100 ppm dosage increased at a faster rate than by other dosages at the third day. Pooling the data from treatments with non-significant differences to the 100 ppm treatment group in order to create a control group with more observations is a scientifically doubtful procedure. Apparently, authors tried to increase the statistical power as an effort to push the difference between the 100 ppm and the pooled control data to a statistically significant level. Lastly, for YS to be effective in this study, one would expect a dose-dependent relationship, which seems to be lacking here. According to the authors, results may be affected by the lower moisture content of the 100 ppm manure (65.8% as opposed to 72% for the other dosages). A lower moisture content could affect microbial activity and thus ammonia volatilization. The 50 or 200 ppm dosages did not result in statistically significant ammonia emission reductions.

In poultry farms, ammonia emissions may partly depend on the pH of the manure; a low acidity is associated with a low ammonia volatilization. YS is able to decrease the pH value of the poultry environment which leads to a decrease of ammonia emission and formation. Its mode of action in ammonia mitigation is believed to be based on modification of the gut microbe population and improvement of digestion and nutrient absorption (Saeed et al., 2018). In this overview, no literature on effects of YS on poultry microbe population were included. Also, growth and immune responses and production performance could be enhanced. These processes could lead to a reduced content of undigested nutrients in poultry feces (Saeed et al., 2018). However, a decreased N-content in manure is beneficial only if the nitrogen is fixated in the body. No information was found on the effects of YS extract on mineral fixation.

In a study by Patoary et al. (2020), 240 broiler chicks were divided into four treatment groups with three replicates of 20 chicks per each. Chicks were treated via their drinking water for four weeks with one of three different concentrations of YS extract (1 mL of YS per 16, 20 or 24 L of water) versus water without YS extract (table 1). Overall, live weight, carcass quality, feed conversion ratio improved most and feed intake increased most at a concentration of 1 ml YS extract per 20 liters of drinking water. After four weeks of treatment, significant ammonia emission reductions from litter were reported in all treatment groups (-55% at 1 ml YS extract per 16 liters of drinking water; -42% at 1 ml YS extract per 20 liters of drinking water; -23% 1 ml YS extract per 24 liters of drinking water). Ammonia concentrations were determined using paper strips that change colour depending on the ammonia concentration, and a color chart for matching the colouring to the ammonia concentration. This measurement method may be accurate, however, it has a low reading precision and produces a value at one given time and location. Moreover, it is not specified if ventilation rates were measured and emissions calculated. As a result, it cannot be concluded whether the ventilation rate was similar between the treatments and whether the ammonia 'emission' data are valid and representative. Due to these issues, the measured values may not represent the emissions of a group in general.

Ayoub et al. (2109) investigated the effects of YS supplementation on litter properties in broilers for five weeks. The broiler chicks were divided into three groups: a control group and two treatment groups with a basal diet with additional YS (Yucca Plus liquid) at a dose of 0.5 ml/l or 1 ml/l drinking water. Litter samples were taken at day 21 and day 35 of the experiment. The treatment groups showed statistically significant lower nitrogen contents (between -12% and -55%), determined using the Kjeldahl method. The moisture content of the litter was also lower in both treatment groups (between 5% and 11%). According to the authors, their findings correspond with results from earlier studies (Cheeke et al., 2009), which they, consequently, attribute to ammonia-binding properties of YS extract.

Cohuo-Colli et al. (2017) investigated the effects of YS supplementation on ammonia emission in broiler chickens for 50 days. Treatments consisted of two types of litter density (1 kg/m^2 and 2 kg/m^2) and a control or 125 ppm YS extract in the form of MicroAid (a commercial product containing 30% YS dissolved solids). In both low and high density litter, ammonia emission was decreased significantly upon YS treatment. According to the authors, the observed effect are due to a promoted feed digestibility by the steroid saponins present in YS extract. However, the study design was poorly documented. Likewise, although the authors refer to ammonia 'emission', ventilation rate measurements are not reported. Therefore, it is unclear whether these results can be interpreted as effects on ammonia emission.

Çabuk et al. (2004) studied the effects of YS supplementation on broiler performance. From the age of 1 day to 21 days, 960 broiler chicks were fed a starter diet. From day 22 to 42, the chicks received one of four different treatment diets, one of which contained 0.120 mg YS/kg. After 42 days, ammonia concentrations and FCR were compared to the control group. FCR did not differ significantly between the control group and the YS treatment group. Ammonia concentration of the broiler house was measured and had decreased significantly after 42 days (-15%). However, the Dräger gas detector pomp used in this study only measures ammonia concentration, ventilation rates were not monitored. Therefore, these results to not represent the effects of YS on ammonia emission.

Lazarevic et al. (2014) used 902 broilers in 12 groups to investigate the effects of YS treatment (500g/t De-Odorase) on animal performance and ammonia concentration. The one-day old birds were housed in individual pens with vertical ventilation, which was switched of during ammonia measurements. Ammonia in the atmosphere was measured at day 14, 28, 32, 37 and 42 at the height of the birds head using Dräger tubes. Only at day 37, a statistically significant decrease in litter ammonia concentration was found. At 42 days, no statistically significant decrease was observed. FCR improved significantly (-13%, $P<0.05$), which according to the authors was due to the reduced feed intake, as body weight was not changed. They suggest that nutrient absorption is enhanced by steroid saponins in YS. Saponin content of De-Odorase product was not documented.

A study from the applicant Jadis Additiva B.V. and HBLFA Raumberg-Gumpenstein (2019) was well designed and documented when compared to the other studies found on YS supplementation. A trial group and a control group of 420 broilers each were kept in separate broiler barns. Barns were ventilated mechanically. Broilers were reared for about 35 days. YS powder was added to the feed of the trial group at a ratio of 125 g per 1000 kg (saponin content ≥ 10.5%). Concentrations of ammonia and carbon dioxide were measured in the outlet and background air. The ventilation rate was monitored to calculate ammonia emissions. The study included four growth cycles, and treatment and control were switched between barns between each cycle. The mean ammonia emissions of treatment/control group respectively, expressed in g/animal place per year, were 15/17 for cycle 1, 47/38 for cycle 2, 17/25 for cycle 3, and 22/38 for cycle 4, equivalent with relative differences of -12%, +24%, -32%, and -42%. The overall mean ammonia concentration was 5.1 ppm for YS treatment versus 5.9 ppm for the control. The overall mean ammonia emission was 25.3 g/animal place per year for YS treatment versus 29.5 g/animal place per year for the control, which is a relative difference of -14%. The authors declare this difference of -14% to be statistically significant (P = 0.0009). However, three aspects stand out with regard to this finding:

1. statistical analysis of the data mentioned is unlikely to result in such very high statistical significance. Further details on the statistical procedure gathered and provided by the applicant made clear that a repeated measurements model was applied to week-averaged emissions. This approach has probably over asked the data. Since repeated measurements within a production cycle are autocorrelated, it is preferred to use the emissions on the level of one cycle as independent observation, for instance in a Paired Samples *t*-test. In order to discriminate a treatment effect, this approach requires at least five to eight independent cycles;
2. graphs of ammonia concentration as a function of time within each cycle show a consistently lower ammonia concentration for the treatment group only in the fourth cycle. In the other three cycles, such pattern is not clearly visible. Lines of treatment and control revolve around each other in time;
3. reductions based on ammonia concentrations deviate substantially from those based on ammonia emissions which means that the ventilation rate must have differed between the two.

These three aspects were not addressed by the authors in their report. Overall, the study results do not convincingly show a lower ammonia emission for the YS treatment. A (small to moderate) reduction effect might be present, but requires a larger study to be detected.

3.3.2 Steers

One study was found on ammonia-related effects of sarsaponin supplementation in steers. Table 3 presents an overview of the study design and outcomes.

Table 3 *Overview of studies on ammonia-related effects of sarsaponin supplementation in steers. Percentages in bold indicate a significant effect of YS treatment.*

Steers	Experimental period	Number of animals	Mean BW	Yucca treatment	Diet	Experimental facility	Method	Ammonia-related effects of YS
Lila et al. (2005)	3 periods of 14 days of adaptation + 4 days digestion trial	3 Holstein steers	248 ± 27 kg	0; 11.2; 22.4 g sarsaponin per 2.25 kg DM (equal to 0.5% and 1% sarsaponin of DM)	Sudangrass hay + concentrate mixture at a ratio 1.5:1 twice daily; and sarsaponin (0, 0.5 and 1% of DM)	Digestion stalls	Ammonia-N determined with micro-diffusion method	Ruminal ammonia-N 5 hrs after feeding: Control: 8.8 mg/dl **0.5% sarsaponin: -6%** **1% sarsaponin: -10%** Ruminal urea-N 5 hrs after feeding: Control: 7.2 mg/100 ml **0.5% sarsaponin: -11%** **1% sarsaponin: -28%**

Lila et al. (2005) studied the effects of sarsaponin on ruminal protozoa count in steers. Sarsaponin was added to the diet (0; 0.5; 1% of DM) of three Holstein steers for four days. After the four-day

digestion trial, rumen fluid and plasma samples were collected and the ammonia-N and urea-N concentrations were measured. A linear and significant decrease in ammonia-N concentration was found at five hours after morning feeding. According to the authors, the effect is likely to result from decreased bacterial lysis, which in turn could be a result of inhibited growth of protozoa. Another suggestion is the inhibition of deamination and direct ammonia binding in the rumen. Sarsaponin supplementation decreased the urea-N concentrations in plasma significantly. This effect was also reflected by the decreased ammonia-N concentration in the ruminal fluid. With only three steers, the number of animals used is very low, and stronger conclusions can be drawn in a study design with more replicates.

3.3.3 Pigs

Four studies were found on ammonia-related effects of YS supplementation in pigs. Table 4 presents an overview of the study design and outcomes.

Table 4 *Overview of studies on ammonia-related effects of YS supplementation in pigs. Percentages in bold indicate a significant effect of YS treatment.*

Pigs	Experimental period	Number of animals	Mean BW	Yucca treatment	Diet	Experimental facility	Method	Ammonia-related effects of YS	
Panetta et al. (2006)	4 days, followed by 3 days of continuous measurements	9 pigs in a cross-over design	41.6 ± 3 kg	0; 62.5; 125 mg of YS extract/kg diet	Lysine supplemented corn-soybean diet (170 g L/kg CP) + 3 different dosages of YS	3 environmentally controlled chambers	Aerial ammonia concentrations of chamber exhaust measured with TEI Model 17C chemiluminescence ammonia analyzer	Ammonia emission via exhaust air: Control: 1.45 ppmv 62.5 mg YS/kg: -9%^{ns} 125 mg YS/kg: -21%^{ns}	
Min et al. (2001)	6 weeks	6 groups with 5 replicates with 4 pigs per replicate	18 ± 0.51 kg	0 or 120 mg YS extract/kg	Iso-caloric (3.36 ME kcal/kg) basal diet containing 0.85; 1.00 or 1.15% lysine and 16; 18; or 20% CP, respectively + 2 different dosages of YS	Concrete floor pen	Colorimetric analysis of fresh feces	Ammonia-N content in feces: Control low CP: 12.1 ml/g feces; with 120 mg YS extract/kg: +26.1^{ns} Control medium CP: 12.0 ml/g feces; with 120 mg YS extract/kg: +10.6%^{ns} Control high CP: 13.7 ml/g feces; with 120 mg YS extract/kg: +36.4%^{ns}	
Colina et al. (2001)	Two 4-week trials and three 5-week trials	3 groups with 5 replicates with 10 pigs per replicate	3-6 kg	0 or 125 ppm YS extract (De-Odorase)	23.5% CP, 1.75% lysine, 0.75% Ca, and 0.70% P, with 165 mg/kg apramycin sulfate + anhydrous calcium chloride or + 2 different dosages of YS	Three identical, environmentally regulated pig nursery rooms	Aerial ammonia measured by Sensidyne aspiration tubes[1] (poin-in-time value) and diffusion tubes[2] (8-h time period); manure ammonia measured by ammonia-gas detecting electrode	Aerial ammonia[1]: Control: 3.6 ppm 125 ppm YS: -23%^{ns} (P = 0.08) Manure ammonia: Control: 588 ppm 125 ppm YS: +27%^{ns}	Aerial ammonia[2]: Control: 8.2 ppm 125 ppm YS: -31%^{ns}
Chen et al. (2021)	60 days	4 groups with 20 sows	Unknown	0; 0.06%; 0.12%; 0.24% YS extract (>10.5% saponin)	66% corn, 20% soybean meal, 10% bran, 4% premix	Single crates and individual farrowing crates	Total nitrogen determined by Kjeldahl method and urea nitrogen determined by commercial kit	Total nitrogen retention in manure at 12h: Control: 3.3% 0.06% YSE: -0%^{ns} **0.12% YSE: -18%** **0.24% YSE: -21%** Urea nitrogen in manure at 24h: Control: 70 mmol/l 0.06% YSE: -7%^{ns} **0.12% YSE: -29%** **0.24% YSE: -24%** Ammonia-N content in manure at 24: Control: 4.7mg/g feces **0.06% YSE: +38%** **0.12% YSE: +62%** **0.24% YSE: +36%** Urease activity at G100: Control: 0.26 0.06% YSE: +0%^{ns} 0.12% YSE: +8%^{ns} 0.24% YSE: -4%^{ns}	At 120h: Control: 2.5% **0.06% YSE: +32%** **0.12% YSE: +40%** **0.24% YSE: +44%** At 120h: Control: 22 mmol/l 0.06% YSE: -18%^{ns} 0.12% YSE: -25%^{ns} 0.24% YSE: -22%^{ns} At 120h: Control: 3.3mg/g feces **0.06% YSE: -18%** **0.12% YSE: -18%** **0.24% YSE: -21%** At L15: Control: 0.34 0.06% YSE: -6%^{ns} 0.12% YSE: +6%^{ns} 0.24% YSE: -9%^{ns}

Panetta et al., (2006) performed two trials feeding nine swine with lysine and different levels of YS extract (table 4). YS was added in the form of De-Odorase (a commercial product based on YS extract); 0 mg/kg, 62.5 mg/kg and 125 mg/kg. The exact YS content of the product was not specified. In this study, ammonia concentrations in the exhaust air were compared between treatment groups which was a valid approach because the air flow rate could be kept similar between the different treatment groups. Supplementation with YS did not affect feed intake, average daily gain or feed efficiency after the 7-day trials. Ammonium and nitrogen concentrations of manure (slurry) and ammonia concentrations were not affected significantly either. Lack of statistically significant results may be due to the small sample size. According to Panetta et al. (2006), it might also be due to the short duration of the study or the age of the pigs used.

A study by Min et al. (2001) used 120 pigs in a six-week trial, with diets varying in protein levels (table 4). After supplementing pigs with YS extract (0 and 120 mg/kg diet) and different concentrations of dietary protein (16, 18 and 20%) on rotation basis. Ammonia-N content in fresh

feces was measured; ammonia concentrations in air or airborne emissions were not determined. The ammonia-N in feces tended to increase when protein level and YS extract supplementation were increased. Ammonia-N content was not significantly affected by different protein levels. The content of ammonia-N in feces increased with 36% upon YS extract addition but these results were not statistically significant. Min et al. (2001) suggest that this might have been caused by the ammonia-binding abilities of YS extract, which could reduce levels of free ammonia. It is surprising that effects on the urea content of fresh pig urine were not included in the study, since ammonia mainly originates from urea in urine and hardly from feces.

Colina et al. (2001) performed two 4-week trials (preliminary study) and three 5-week trials (major study) with 150 pigs (table 4). The pigs received diets with 125 ppm YS extract from De-Odorase. The saponin content of De-Odorase was not documented. Aerial ammonia concentrations were measured in two ways: in samples from the centre of the room by aspiration tubes and in different places in the room by diffusion tubes. Ammonia concentrations were also determined in manure (slurry) samples. No statistically significant effects of YS supplementation were found. Although the study found no significant effects, the authors still attribute the unsignificant reduction in aerial ammonia concentrations to the glyco-components of YS extract. However, since ammonia hardly originates from feces, it is difficult to interpret these results.

Chen et al. (2021) investigated the effects of YS extract supplementation in sow during late gestation and lactation. In four treatment groups, with 20 sows in each group, YS extract was supplemented from day 80 of gestation to day 21 of lactation. Treatment doses were 0.0% (control), 0.06%, 0.12% and 0.24% YS extract, which contained >10.5% saponin. Daily, two fresh fecal samples were taken for nutrient digestibility measurements. A fresh feces and urine mixture was analysed for its total-N and urea-N content. Results from the 0.06 group showed significantly higher digestibility of both dry matter ($P=0.04$) and fat ($P=0.03$). Only between 0-24 hours of manure storage, urea-N reduced significantly in the 0.12 and 0.24% groups compared with the control group. Likewise, total-N content in manure was significantly lower in the 0.12 and 0.24% groups between 0-24 hours of storage. Combining these observations, the authors suggest that protein utilization could be improved during gestation and lactation with doses of 0.12 and 0.24% YS extract. However, after 48-120 hours of storage, the effect of these doses seemed to be reversed and the total-N content in manure was actually significantly increased. Total loss of ammonia-N was measured over time and was significantly lower in YS treatment groups, during both gestation and lactation. Chen et al. suggest that in these groups, higher levels of ammonia-N were kept in the manure and could not be emitted as ammonia. Moreover, because urease activity was not significantly changed, the authors suggest that ammonia nitrogen is entrapped in the manure by YS extract, which reduces ammonia emission during storage.

3.3.4 Dairy cows

Five studies were found on ammonia-related effects of YS supplementation in dairy cows. Table 5 presents an overview of the study design and outcomes.

Table 5 *Overview of studies on ammonia-related effects of YS supplementation in dairy cows. Percentages in bold indicate a significant effect of YS treatment.*

Dairy cows	Experimental period	Number of animals	Mean BW	Yucca treatment	Diet	Experimental facility	Method	Ammonia-related effects of YS
Wilson et al. (1998)	21 days	4 groups of 3 cows	640 kg	0 or 9 g YS/day	70% DM; 20% CP with low soluble protein (SP 34.4%) or high SP (44.9%) + 2 different dosages of YS	Individual tie stalls	Enzymatic assay[1] and mid infrared reflectance spectroscopy[2]	Ruminal ammonia-N concentration: Control: 16.70 mg/dl Low SP: +6%[ns] High SP: no difference[ns] Urea N concentration in plasma: Control: 17.20 mg/dl Low SP: -6%[ns] High SP: + 1%[ns] Urea N concentration in milk[2]: Control: 15.27 mg/dl; Low SP: -2%[ns]; High SP: + 1%[ns] Urea N concentration in milk[2]: Control: 19.39 mg/dl; Low SP: -1%[ns]; High SP: -1%[ns]
Holtshausen et al. (2009)	3 28-day periods	2 groups of 6 cows	627 ± 55 kg	0 or whole-plant YS powder at 10 g/kg of DM; YS powder contained 6% saponin	Control diet (52.1% DM; 17.0% CP) 51:49 forage: concentrate ratio + Q. saponaria or + 2 different dosages of YS	Individual tie stalls fitted with rubber mattresses and bedded with wood shavings; environmental chambers for methane production measurement during last week of period	Measured in environmental chambers and with sulfur hexafluoride (SF6) tracer technique	Net ruminal ammonia-N production: Control: 7.62 mg/dl 10g/kg YS: -12%[ns]
Abdel-Raheem et al. (2019)	4 months, of which 14 days digestibility trial	3 groups of 5 cows	167 ± 3.5 kg	0; 1 g YS powder/kg DM in concentrate mixture; 2 g YS /kg	Concentrate mixture (2% of BW; 14.75% CP), wheat straw (2.76% CP), Egyptian clover (17.64% CP), roughage level 1% of BW + 3 different dosages of YS	Separate pens with concrete floor and equipped with locally manufactured feed manger	Method 973.49 of AOAC	Ruminal ammonia-N concentration: Control: 15.51 mg/dl **1g YS/kg DM: -42%** **2g YS/kg DM: -57%**
El-Din et al. (2008)	Not reported	4 groups of 3 male + and 3 female Friesian calves	119.43 (male) and 117.32 kg (female)	0; 120; 160; 200 g YS extract/ton concentrate feed mixture	DM basis of 60% concentrate feed mixture + 20% berseem hay + 20% rice straw with 0; 120, 160 or 200 g YS extract (Ammonase-300) supplementation / ton concentrate feed mixture	Not documented	Ammonia-N determined using saturated solution of magnesium oxide distillation according to AOAC method, urea-N determined calorimetrically	Ruminal ammonia-N concentration: Control: 18.51 mg/100 ml **120g YS/ton: -5%** **160g YS/ton: -10%** **200g YS/ton: -13%** Blood plasma urea-N concentration: Control: 34.38 mg/dl **120g YS/ton: -5%** **160g YS/ton: -7%** **200g YS/ton: -9%**
Hristov et al. (1999)	14 days of adaptation + 14 days of sample collection	6 heifers	443 ± 6.1 kg	0; 20; 60 g YS powder/day	Alfalfa silage:barley grain-based diet	Not documented	Ammonia determined in supernatant (Broderick & Kang, 1980)	Ruminal ammonia concentration: Control: 6.29 mM 20g YS: -11%[ns] 60g YS: -21%[ns]

In a study by Wilson et al. (1998), 12 dairy cows were fed with 0 or 9 g/day of YS extract with different levels of soluble protein. Ruminal ammonia concentrations were measured. Urea N concentrations were determined in plasma, as well as in milk. Both variables reflect the efficiency of N-utilisation from feed and are proxies for N-excretion as urea in urine, and, subsequently, ammonia emission from urine puddles. No statistically significant effects of YS supplementation were found on DM intake, ammonia-N in ruminal fluid, plasma urea N and milk urea N. No significant interaction between soluble protein levels and YS supplementation could be observed.

In a cross-over experiment by Holtshausen et al. (2009), two groups of six cows were treated with either a control or a saponin source, one of which was YS powder. YS was added to the diet at a concentration of 10 g/kg DM. No significant effects on ammonia-N concentrations in ruminal fluid were observed among treatments. The authors state that this could be due to adaption of rumen microorganisms; although no significant correlation between treatment and day was found. Lack of effects on ammonia N concentrations are consistent with the lack of effects on ruminal protozoa count. The small sample size may also have contributed to the lack of significant effects. In more reviews, the lack of effects is thought to be associated with adaptation of rumen microbial population of the animals. This suggest that the effect of YS supplementation may be only temporary as the microbial population could be able to adapt to this treatment (Das et al., 2012; Beauchemin et al., 2009).

Abdel-Raheem et al. (2019) fed 15 buffalo cows with a control, 1 or 2 g YS powder/kg DM. The YS powder contained 6% of saponins. The experiment lasted four months, while the digestibility trial lasted for only fourteen days, during which rumen contents were collected four hours after each morning feeding. They found a significant effect of both dosages of YS powder on ruminal ammonia-N concentrations (1g YS/kg DM: -42% and 2g YS/kg DM: -57%). The authors state that this was caused by the effect of saponins on ruminal protozoal numbers, which were also significantly reduced by supplementation with YS.

El-Din et al. (2008) studied the effects of YS supplementation on productive performance of growing calves. In total, four groups of 7 male and 7 female calves were formed, in which 3 male and 3 female calves were assigned to one of the four treatments (control group; 0; 120; 160 or 200 g YS extract). With increasing levels of YS supplementation, the ruminal ammonia-N concentrations decreased

significantly. Urea-N concentration was measured in blood plasma and decreased significantly with increased YS dosages as well. However, the study design was not very well documented. Some strong limitations of this study are that both the saponin content of the YS extract as well as the duration of the feeding trial were not documented. Therefore, interpretation of the results is difficult.
In a study by Hristov et al. (1999), 6 heifers were fed 0 (control), 20 or 60 g YS powder (dried and pulverized whole plant) per day. Saponin content of YS powder was not reported. Duration of the experiment was 14 days of adaptation to treatment followed by 14 days of sample collection. Average ruminal ammonia concentrations fluctuated and were not affected significantly by YS treatment. A large range of ruminal ammonia concentration was observed (minimum 0.12 and maximum 18.2 mmol/L). The authors expected the glycofractions in YS to bind ammonia and bacterial lysis to decrease. Hristov et al. (1999) suggest that the lack of significant results could be explained by the dosages used and the time of sampling.

3.3.5 Sheep

Three studies were found on ammonia-related effects of YS supplementation in sheep. Table 6 presents an overview of the study design and outcome.

Table 6 *Overview of studies on ammonia-related effects of YS supplementation in dairy sheep. Percentages in bold indicate a significant effect of YS treatment.*

Sheep	Experimental period	Number of animals	Mean BW	Yucca treatment	Diet	Experimental facility	Method	Ammonia-related effects of YS
Yurtseven et al. (2018)	17 days including 10 days of adaptation	4 groups of 4 dairy ewes	50.4 ± 1.02 kg	0 or 1.5 kg YS per tonne of the ratio	TMR containing mixture of a concentrate and lucerne hay (89% DM; 15,6% CP) + 2 different dosages of YS	Individual pens with no bedding	Gas measurements by filtration	N output from manure: Control: 1.8 ppm/ml 1.5 kg YS/tonne: no difference[ns]
Gumus et al. (2016)	10 days of adaptation + 10 week fattening period	3 groups of 4 replicates with eight lambs per replicate	23.164 ±1.42 kg	0; 100; 200 ppm YS powder (Ekomix Yucca)	Basal ration (DM content: CP: 3.1, CA: 6.69, NDF: 77.45, ADF: 50.32).	Two animals per compartment	Automatic analyzer using commercial test kits (Cobas 8000 analyzer, Roche).	Serum ammonia concentration: Control: 51.571 µg/dl **100 ppm YS: +46%** **200 ppm YS: +18%**
Santoso et al. (2004)	14 days, including 8 days adaptation and 6 days of sample collection	4 sheep	54.5 ± 5.4 kg	0; 240 ppm YS powder	Basal diets comprising timothy silage and concentrate (85:15) (S), and timothy hay and concentrate (60:40) (H), on DM basis (14.8% CP)	Individual cages	Not reported	Ruminal ammonia-N: Control (silage): 248.2 mg/L **240 ppm YS: -8%** Control (hay): 260.6 mg/L **240 ppm YS: -6%**

Yurtseven et al. (2018) conducted a study using 16 dairy ewes assigned to different treatments. A basal diet with 1.5 kg YS extract per tonne of the ratio was compared to the control diet. Although feed intake, live weight or feed conversion ratio were not affected, supplementation with YS extract led to significantly lower N concentration in fresh feces (-34%).

Gumus et al. (2016) studied the effects of YS powder on animal performance and blood parameters in lambs. Using 24 2.5-month old lambs, YS was supplemented at a dosage of 0 (control), 100 ppm or 200 ppm for 10 weeks during the fattening period. Interestingly, they found a significant increase in serum ammonia concentration in both treatment groups (100 ppm: +46% and 200 ppm: +18%). The observed effect was not further explained or discussed by the authors. The saponin content of the YS powder was not reported.

Santoso et al. (2004) used four Cheviot wethers with ruminal fistulas to study the effect of YS powder supplementation. Two control groups were used, of which one was fed a silage-based diet, and the other group was fed a hay-based diet. In the treatment groups, 240 ppm YS was added to both diets. Compared to their own control group, YS treatment groups showed significantly lower ruminal ammonia-N levels (-8% in silage and -6% in hay). This effect was suggested to be the result of the binding capability of glycofraction in YS, which slows down the release of ammonia. Another reason suggested by the authors is that YS saponins affect rumen protozoa count, or that reduced protozoa numbers lead to reduced deamination of amino acids from the diet. However, the number of animals used was very low and should be increased for better interpretation of the results.

3.3.6 Goats

One study was found into ammonia-related effects of saponin supplementation in goats. Table 7 presents an overview of the study design and outcome.

Table 7 *Overview of studies into ammonia-related effects of saponin supplementation in goats. Percentages in bold indicate a significant effect of YS treatment.*

Goats	Experimental period	Number of animals	Mean BW	Yucca treatment	Diet	Experimental facility	Method	Ammonia-related effects of YS
Santoso et al. (2007)	4 periods of 14 days experiment + 8 days of adaptation + 1 day of sampling	4 Kacang goats	20.3 ± 2.78 kg	0; 13; 19.5; 26 mg saponin/kg BW	Elephant grass silage and grain-based concentrate (70:30 on a DM basis)	Individual metabolism cages	Sub-samples of filtrate added to 5ml of 20 ml/l (w/v) NaCl, micro-diffusion method	Ruminal ammonia-N: Control: 161 mg/l **13 mg/kg BW saponin: -26%** **19.5 mg/kg BW saponin: -28%** **26 mg/kg BW saponin: -32%**

In the study by Santoso et al. (2007), goats were used to study the effects of saponins on the ammonia concentration in rumen fluid. Rotating the goats, four doses of saponin were used (0; 13; 19.5; 26 mg saponin/kg BW). Ammonia-N concentrations were measured hourly for four hours after morning feeding. Effects on the longer term were not measured. With all treatments, a peak in ruminal ammonia-N was observed after 1 hour after feeding, followed by a gradual decrease (up to -32%). In this study, ammonia-N concentrations decreased significantly with increasing doses of saponin. The authors suggest three explanations for this effect: it could be due to inhibited growth of protozoa, binding of ammonia to saponin, or 'increased incorporation of ammonia, peptide or amino acids into microbial protein'. A weakness of this study is the number of animal used, with only one goat per treatment and four replicates.

3.3.7 *In vitro* studies

One study was found studying ammonia-related effects of YS supplementation *in vitro*. Table 8 presents an overview of the study design and outcome.

Table 8 *Overview of studies on ammonia-related effects of YS supplementation in vitro. Percentages in bold indicate a significant effect of YS treatment.*

In vitro	Experimental period	Number of animals	Mean BW	Yucca treatment	Diet	Experimental facility	Method	Ammonia-related effects of YS
Holtshausen et al. (2009)	24 hours	Ruminal fluid from 3 Holstein dairy cows	617 ± 8.9 kg	0; 15 g YS extract/kg of substrate DM; 30 g YS/kg of substrate DM; 45 g YS/kg of substrate DM; YS powder contained 6% saponin	Barley silage-based TMR (16.7% CP, 34.4% NDF) with 51:49 forage:concentrate ratio + 4 different dosages of YS	n.a.	Acidification with metaphosphoric acid and centrifugation of samples	Ammonia-N concentration: Control: 26.5 mg/dl **15g YS /kg: -72%** **30g YS/kg: -101%** **45g YS/kg: -100%**

Holtshausen et al. (2009) performed an *in vitro* study using different doses of YS powder. The ruminal fluid that was used originated from three Holstein dairy cows. After obtaining the fluid, YS was added at a dose of 0, 15, 30 or 45 g/kg of YS powder containing 6% saponin. The study showed a linear and statistically significant decline of ammonia-N concentration with increasing levels of saponin. At the 30 and 45 g-dose, the ammonia-N concentration in the ruminal fluid reached a zero-level.

3.4 Studies on methane-related effects

3.4.1 Dairy cows

One study was found on methane-related effects of YS supplementation in dairy cows. Table 9 presents an overview of the study design and outcome.

Table 9 *Overview of studies on methane-related effects of YS supplementation in dairy cows. Percentages in bold indicate a significant effect of YS treatment.*

Dairy cows	Experimental period	Number of animals	Mean BW	Yucca treatment	Diet	Experimental facility	Method	Methane-related effects of YS
Holtshausen et al. (2009)	3 28-day periods	2 groups of 6 cows	627 ± 55 kg	0 or 10 g YS extract/kg of DM	CON diet (52,1% DM; 17.0% CP) 51:49 forage:concentrate ratio + Q. saponaria or + 2 different dosages of YS	Individual tie stalls fitted with rubber mattresses and bedded with wood shavings; environmental chambers for methane production measurement during last week of period	Measured in environmental chambers and with sulfur hexafluoride (SF6) tracer technique	Methane production in chambers: Control: 19.2 mg/dl 10g/kg YS: -1%[ns]

In the same experiment by Holtshausen et al. (2009) mentioned earlier (par. X), two groups of six cows were fed either a diet supplemented with a saponin source or a control diet to assess effects on enteric methane emission in respiration chambers. One of the saponin sources was YS additive which was added at a concentration of 10 g/kg DM. Methane emission was measured in environmental chambers for three consecutive days. These methane concentrations were done by a methane analyser in the ingoing and exhaust air ducts. Additionally, methane emission was measured using SF6 tracer technique. These measurements were done for five consecutive days and differed 16% from the environmental chamber methane measurements. None of the measurements showed statistically significant effects of YS supplementation on methane emission. The authors link the lack of effect to the possible adaptation of rumen protozoa; however, no correlation between treatment and day was found.

3.4.2 Sheep

One study was found on methane-related effects of YS supplementation in sheep. Table 10 presents an overview of the study design and outcome.

Table 10 *Overview of studies on methane-related effects of YS supplementation in dairy sheep. Percentages in bold indicate a significant effect of YS treatment.*

Dairy ewes	Experimental period	Number of animals	Mean BW	Yucca treatment	Diet	Experimental facility	Method	Methane-related effects of YS
Yurtseven et al. (2018)	17 days including 10 days of adaptation	4 groups of 4 ewes	50.4 ± 1.02 kg	0 or 1.5 kg YS per tonne of the ratio	TMR containing mixture of a concentrate and lucerne hay (89% DM; 15,6% CP) + 2 different dosages of YS	Individual pens with no bedding	Gas measurements by filtration	Methane emission from manure: Control: 1.8 ppm/ml 1.5 kg YS/tonne: no difference[ns]

Results of the study by Yurtseven et al. (2018) showed that the digestibility of organic matter was significantly lower in dairy ewes supplemented with 1.5 kg YS extract per tonne of the ration (0.80% in the control group; 0.63% in the treatment group). Feed intake, live weight or feed conversion ratio were not affected. Manure output was almost doubled compared to the control diet but so far, no literature was found confirming a relationship between manure output and saponins present in YS extract. DM of the manure did not differ significantly between groups. The authors suggest a link between higher manure production and high acid detergent fibre (ADF) content of the manure and low ADF digestibility in the treatment diet. However, although possible explanations have been considered, it remains unclear how this increase in manure production was caused. YS supplementation did not have any effect on total gas emission or methane emission from slurry. Daily emissions of methane were not affected. However, the authors reported that they had some difficulties during separating urine from manure, and during sampling the manure. As a result, the samples were stored in an oxygen-rich environment. Under these aerobic circumstances, methane-producing bacteria may have been inhibited. Also, the amount of urine in the samples was very low as the faeces of the sheep was compressed and pelletized. These issues may have affected the results.

3.4.3 Steers

One study was found studying methane-related effects of sarsaponins in steers. Table 11 presents an overview of the study design and outcome.

Table 11 *Overview of studies on methane-related effects of YS supplementation in steers. Percentages in bold indicate a significant effect of YS treatment.*

Steers	Experimental period	Number of animals	Mean BW	Yucca treatment	Diet	Experimental facility	Method	Methane-related effects of sarsaponins
Lila et al. (2005)	3 periods of 14 days of adaptation + 4 days digestion trial	3 Holstein steers	248 ± 27 kg	0; 11.2; 22.4 g sarsaponin per 2.25 kg DM (equal to 0.5% and 1% sarsaponin of DM)	Sudangrass hay + concentrate mixture at a ratio 1.5:1 twice daily; and sarsaponin (0, 0.5 and 1% of DM)	Digestion stalls	Gas measurement by infrared gas analyzer detector	Rate of methane production: Control: 29.8 L/d.kg of DMI **0.5% sarsaponin: -8%** **1% sarsaponin: -13%**

Lila et al. (2005) studied the effects of sarsaponin on ruminal fermentation and methane production in steers. Sarsaponin was added to the diet (0; 0.5; 1% of DM) of three Holstein steers for four days. After this digestion trial, methane production was measured for four days. A significant and linear reduction of methane production upon sarsaponin was found. This effect remained until the last day of

measurement, so whether ruminal adaptation to sarsaponin would occur on the longer term could not be said. The authors suggest that methane production is probably inhibited by inhibition of H2-producing bacteria, or other bacteria using pyruvate-ferredoxin oxireductase. However, a weakness of this study is the number of animals that were used, as it is very low.

3.4.4 *In vitro* studies

Two studies were found studying methane-related effects of YS supplementation *in vitro*. Table 12 presents an overview of the study design and outcome.

Table 12 *Overview of studies on methane-related effects of YS supplementation in vitro. Percentages in bold indicate a significant effect of YS treatment.*

	Experimental period	Number of animals	Mean BW	Yucca treatment	Diet	Experimental facility	Method	Methane-related effects of YS
In vitro								
Holtshausen et al. (2009)	24 hours	Ruminal fluid from 3 Holstein dairy cows	617 ± 8.9 kg	0; 15 g YS extract/kg DM; 30 g YS/kg DM; 45 g YS/kg DM	Barley silage-based TMR (16.7% CP, 34.4% NDF) with 51:49 forage:concentrate ratio + 4 different dosages of YS	n.a.	Gas production measurement using gas–liquid chromatography	Methane concentration in headspace gas: Control: 27.1 mg/g of DM **15g YS/kg: -9%*** **30g YS/kg: -16%*** **45g YS/kg: -26%***
Xu et al. (2010)	24 hours	Ruminal contents collected from two ruminally-fistulated steers	Unknown	0 or 110 mg YS extract/kg	700 g/kg alfalfa hay + 300 g/kg of corn-based concentrate; all forage diet, medium forage diet (0.5 forage:0.5 concentrate) or low forage diet (0.1 forage:0.9 concentrate) + 2 different dosages of YS	n.a.	Gas chromatography	Forage-based diet methane production in gas sample: Control alfalfa: 9.16 ml; **+ YS: -10%*** Control fescue: 8.69 ml; **+ YS: -5%*** Control orchard: 8.08 ml; **+ YS: -12%*** Control bermuda: 6.28 ml; **+ YS: -14%*** Control switch: 5.41 ml; **+ YS: -3%*** Medium-forage diet methane production in gas sample: Control alfalfa: 9.56 ml; **+ YS: -7%*** Control fescue: 8.84 ml; **+ YS: -7%*** Control orchard: 9.12 ml; **+ YS: -11%*** Control bermuda: 8.69 ml; **+ YS: -6%*** Control switch: 7.24 ml; **+ YS: -4%*** 90% concentrate diet methane production in gas sample: Control alfalfa: 12.00 ml; **+ YS: -9%*** Control fescue: 12.73 ml; **+ YS: -12%*** Control orchard: 11.64 ml; **+ YS: -8%*** Control bermuda: 11.35 ml; **+ YS: -8%*** Control switch: 11.52 ml; **+ YS: -6%***

In the *in vitro* experiment by Holtshausen et al., (2009) which was also discussed in par. X, the effects of increasing doses YS on methane production and side effects on ruminal fermentation and fibre digestion were investigated. This was done using ruminal fluid from dairy cows that was incubated with either a control or one of the three doses of saponin additive. Gas production was measured at multiple points in time for 24 hours using a water displacement technique. Total gas production in the headspace gas of the samples was measured after 0, 2, 6, 12 and 24 hours. Methane production decreased significantly after saponin treatment. This decrease was partially attributed to the lower *in vitro* NDF digestibility by the authors, which were significantly lower at all YS dosages (between 17% at 15 g YS/kg and 50% at 45 g YS/kg).

In the study by Xu et al. (2010), different feedstuffs were used for in vitro fermentations. Forages used were alfalfa, fescue, orchard grass, bermuda and switch grass without YS or with the addition of 110 mg/kg YS extract. The forages were used in three different diets: a complete (100%) forage diet, medium (50%) forage diet and a low (10%) forage diet. Methane proportion and production

decreased consistently and significantly with YS extract supplementation in all forage types. There was no interaction between YS extract and forage source. The authors suggest that there is an association between methane reductions with protozoal numbers and inhibited H2 production. The latter effect was thought to be due to inhibition of specific microbes caused by steroidal saponins. However, neither protozoal numbers or H2 production was measured in this experiment by Xu et al. (2010).

In their meta-analysis, Jayanegara et al., (2014) reported a decrease of *in vitro* ruminal methane emission as a result of increasing exposure to saponin-rich sources. According to the authors, this could be seen as a genuine effect, despite a large diversity of saponin structures.

3.5 Studies on odour-related effects

3.5.1 Poultry

Two studies were found on odour-related effects of YS supplementation in poultry. Table 13 presents an overview of the study design and outcome.

Table 13 *Overview of the studies on odour-related effects of YS supplementation in poultry. Percentages in bold indicate a significant effect of YS treatment.*

Broilers	Experimental period	Number of animals	Mean BW	Yucca treatment	Diet	Experimental facility	Method	Effects of YS on odour
Amon et al. (1997)	7 weeks	4 groups of 8000 broilers	Unknown – 2.03 kg at end of trial	0 or 165 g De-Odorase/tonne of feed. YS concentration unknown	Unknown diet + 2 different dosages of YS	2 sites with almost identical buildings with 2 rooms	Olfactometric measurements of total airflow and velocity exhausted from each room	Odour concentration/odour emission rate from exhaust air: Control: 2300 ou/m^3 165 g De-Odorase: + 8.26%^{ns}
Raumberg-Gumpenstein (Jadis Additiva B.V. 2019)	29 days	2 groups with 2 replicates with 210 birds per replicate	Mean slaughter weight 2117.4 g	125 g per 1000 kg (saponin content ≥10.5%)	Starter = 22.5% CP; midway feeding phase = 21.5% CP; final fattening phase = 21.5% CP	Two barns with two sections, groups were rotated after each cycle	Olfactometer TO 8, ecoma	Average odour emission in exhaust air: Control: 513.50 ou/m^3 **0.125 g YS/kg: -6%**

A study by Amon et al. (1997) was conducted to investigate effects of De-Odorase on odour emission from broilers. Groups of 8000 birds were fed either a control feed or feed containing 165mg De-Odorase/kg. The product contained selected glycocomponents from YS, however, the exact composition of the product is not reported. Also, average body weight of the broilers at the start of the experiment and basal diet composition were not documented. No effects on feed conversion ratio or live weight gain were seen. The authors suggest that the preparation may reduce odour and ammonia emissions, however, no statistically significant effects on odour concentration or ammonia emission were seen. Exact numbers of odour emission were not documented.

A study from the applicant Jadis Additiva B.V. and HBLFA Raumberg-Gumpenstein (2019) was well designed and documented when compared to the other studies found on YS supplementation. A trial group and a control group of 420 broilers each were kept in separate broiler barns. Barns were ventilated mechanically. Broilers were reared for about 35 days. YS powder was added to the feed of the trial group at a ratio of 125 g per 1000 kg (saponin content ≥ 10.5%). Concentrations of odour were determined by olfactometry in air samples taken from the exhaust on days 8, 15, 22, and 29 in the growth cycle. The ventilation rate was monitored to calculate odour emissions. The study included four growth cycles; treatment and control were switched between rooms between each cycle. The mean odour emissions of treatment/control group respectively, expressed in OU/s per Livestock Unit, were 53/60 for cycle 1, 176/185 for cycle 2, 239/192 for cycle 3, and 114/202 for cycle 4, equivalent with relative differences of -12%, -5%, +24%, and -44% (overall average: -9%). The authors report no overall statistically significant difference. It should be noted however, that odour concentration values have a relatively high uncertainty and that four cycles might nog have been sufficient to demonstrate an effect on odour emission when actually present.

4 Conclusions

A mode of action of YS is believed to be mainly attributable to the presence of saponins. The literature on YS extracts suggests that it may alter the microbial composition in the rumen, enhance nitrogen efficiency in the gastro-intestinal tract, inhibit urease activity (the enzyme converting urea into ammonia), bind ammonia, and lower the pH value of manure. However, results from currently available literature on these working mechanisms vary and are incomplete.

In total, one study in laying hens, six studies in broilers, four studies in pigs, five studies in dairy cows, one study in steers, three studies in sheep and one study in goats (overall number of 21) were found investigating effects of YS supplementation on ammonia emission-related variables (ammonia concentration in air, ammonia emission from the building, milk urea concentration, plasma urea concentration, urea-N in slurry, total-N in slurry, et cetera). In only three out of 21 studies, concentrations and emissions of ammonia were directly determined: a vessel experiment using manure from hens previously fed YS (Chepete et al., 2012), a barn experiment in broilers (HBLFA Raumberg-Grumpstein, 2019) and a climate chamber experiment in pigs (Panetta et al., 2016). None of the three studies presents consistent and plausible reductions of ammonia. Although statistically insignificant, the results from some of the studies altogether might be indicative for a small (<20%) reduction.

In total, one study in dairy cows, one study in dairy ewes, one study in steers, and two *in vitro* studies were found investigating the effects of YS treatment on methane emission-related variables (methane concentration in headspace gas, methane emission from manure and in environmental chambers). In the two *in vitro* studies, a statistically significant effect (-9.6%) of YS supplementation on methane concentration was found. In the three *in vivo* studies, statistically significant effects were reported only for the study in steers (-8%, -13%).

Two studies were found investigating the effects of YS supplementation on odour emission in broilers. Both studies found no statistically significant differences in odour between YS-treated and control groups.

General conclusion

On the basis of three suitable studies on ammonia emission (in laying hens, broilers, and pigs), and five suitable studies on methane emission (in dairy cows, dairy ewes, steers and *in vitro*), we conclude that it is unlikely that YS supplementation substantially reduces emissions of those two gases (i.e., beyond 20%) in livestock settings. The results from some of the studies together might be indicative for a small reduction of ammonia emission.

Two suitable studies on odour emission in broilers both report no effect of YS supplementation on odour emission in that animal category: more studies in other animal categories are needed before conclusions on odour emission in general can be drawn.

The aforementioned conclusions are drawn with caution. Firmer conclusions cannot be drawn from the studies because of their low number, moderate quality, and heterogeneity.

As stated in the general conclusion above, firmer conclusion cannot be drawn from the studies because of the low number of studies, their moderate quality, and their heterogeneity. In general, five aspects are identified as omissions in the studies and require more attention in future research. First, in almost all studies, 'raw concentrations' were compared between treatments, whereas multiplying those concentrations with ventilation rates to derive emissions (from ventilated buildings) or productions (e.g. from vessel experiments) are needed for accurate determination of effects. Comparison of concentrations is only valid when ventilation rates are kept identical across treatment groups, but such information was never included in the methodology sections of the articles studied.

Second, multiple studies included low numbers of replicates in the study design, which limits their statistical power (i.e., the chance of finding a statistically significant effect given that the effect is truly present). Third, some studies used very basal measurement methods, unsuited for accurate estimation of concentrations. Fourth, in a number of studies essential information on the procedures required to interpret and evaluate the results is missing. Lastly, most studies reported a dose of YS extract given, instead of a dose of actual saponins. Since saponin content can vary substantially between extracts as a result of differences in plant sources and isolation procedures, it remains unclear how much active components were actually dosed in studies. In addition, most studies investigated the effect of YS extract whereas the applicant, Jadis Additiva B.V., uses YS powder, which efficacy, according to the applicant, may be different. Despite the shortcomings in the assessed literature, we would have expected more or stronger indications of a reduction in emissions in the case YS would have the potential for substantial emission reduction.

At present, no risk assessment by the European Food Safety Authority (EFSA) is available. Such assessment, including data on the efficacy of YS supplementation, is needed for YS to become available as functional feed additive in livestock.

5 Recommendations

This literature study did not provide convincing evidence for the efficacy of YS to reduce emission of ammonia, methane, or odour from animal production. Nonetheless, the combination of some studies might be indicative for some ammonia reduction. In the Conclusions chapter, five major omissions present in the literature studied have been listed which hamper drawing sharp-cut conclusions from that literature. In order to be able to draw reliable and firm conclusions on the potential of YS to reduce gaseous emissions from livestock, research is needed that that fulfils the omissions listed:

- studies must have a sufficient number of replicates in order to determine the statistical significance of treatment effects with sufficient statistical power;
- studies must use high-end, accurate measurement methods for both gas concentrations and ventilation rate;
- effects of YS should be based on comparing gaseous emissions or productions instead of comparing raw concentrations, i.e., ventilation rates must be monitored and accounted for across experimental units;
- studies must include a comprehensive documentation of study design, procedures and details;
- studies must take the dose of actual saponins instead of raw YS extract as the basis for creating treatments and report results in terms of those saponin doses.

Only after such research has become available, reliable and firm conclusions can be drawn on the potential of YS to reduce gaseous emissions from livestock.

A concrete way forward could be to develop a stepwise 'research and development' approach, starting relatively simple and relatively inexpensive at lab scale in well-defined and controlled conditions, and eventually ending in real-life *in vivo* trials. The first steps are suggested to focus on the effects of YS on key variables in metabolism, excretion, production, and volatilisation of ammonia and/or methane. This could be done for one or multiple most promising or relevant animal species. Questions to be answered could include:

- inside which animal species (e.g. monogastric versus ruminants) could YS be applied;
- which dose must be given in which stage of the animal's life and in which frequency;
- on which steps in the (microbiological, chemical and physical) formation processes of pollutant gases does YS intervene and in which way;
- are there any interactions between YS and other variables with regard to its efficacy, such as animal breed, production level or feed ration, that must be taken into account?

Et cetera. The results of each step, including the influence of characteristics in the selected conditions, should be used to decide on the perspectives and questions to be addressed in the next step. Large scale or *in vivo* studies are recommended as a final step of this stepwise approach, provided that earlier steps have indicated realistic perspectives that reward the investment. Such research will also be needed for application of YS as EU feed additive with specific function claims as well.

On the basis of this report, it has been decided in the project to proceed with testing the YS additive at lab scale in well-defined and controlled conditions. Results from these tests will be published in future.

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