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A N I N T E R N A T I O N A L J O U R N A L

Effects of *Yucca schidigera* on gas mitigation in livestock production: A review

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ABSTRACT

Yucca schidigera extract (YSE) has received much interest in the application of manure deodorization and hazardous gas mitigation in livestock rearing conditions. The main objective of this review article was to summarize the current knowledge regarding YSE towards its gas mitigation from livestock excrement. Saponins have been considered to be vital components of YSE in odor control and gas reduction in intensive farming industry due to their potentials in lowering methane for ruminants and ammonia for monogastric animals. This review article mainly covered the studies in ruminants, especially focused on in vitro environment. It also summarized possible reasons of the conflicting results among studies from the perspective of experimental design such as incubation time (in vitro) or storage time of manure, and some other factors such as feed source and dietary composition. In addition to traditional dietary inclusion of YSE alone, recent studies prone to apply YSE in new ways such as combining it with other natural compounds or using it to treat manure directly. Until now, there are still controversies in terms of the effectiveness of YSE in intensive-farming environment among researches, therefore further deeper studies on the expression of YSE bioactivity are needed, especially on the molecular level.

Key words: *Yucca schidigera*, livestock, ammonia, methane, ruminal fermentation

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INTRODUCTION

Increasing attention has been placed on greenhouse gas (GHG) emissions in recent years with deteriorating scenario of global warming nowadays [1,2]. Appropriately 7-18% of the global GHG emissions are originated from livestock sector [3]. Each year domestic livestock can generate 80-115 million tons of methane (CH_4) on a global basis, which is equal to 15-20% of total anthropogenic methane [4]. As another gas generated from livestock, ammonia (NH_3) is harmful to environment as well [5,6], although not labeled as GHG [7]. A statistics showed that agriculture industry took dominant part in ammonia emissions [8], which the main results were coming from barns (34-43%) and livestock waste storage (22-26%) [9].

Several measures have been undertaken to tackle environmental challenges in animal husbandry operations [10], and it was found that the additives such as fenugreek and cinnamomum verum [11] in diets is one of the effective ways to mitigate GHG emissions and ammonia concentrations in livestock rearing process. Compared with chemical feed additives such as antibiotics and ionophores which were considered to be not suitable in the application of feed additives [12], plant-derived substances have more integrated benefits [13]: they can be incorporated as growth promoters (probiotics and prebiotics) [14], or show anti-inflammatory, anti-fungal, anti-infectious or antitoxigenic potentials in livestock production [15,16]. Therefore, phytogenic feed additives are the renewed hotspots of researchers who were aiming at finding their potential values in improving rearing conditions of farming animals in addition to aforementioned benefits [17-19]. Saponins contained in plants, when labeled as feed additives, are one group of phytochemicals that have been studied continuously. This article exclusively discussed more recent studies of a typical saponin-containing plant - *Yucca schidigera*, and its effects on environmental control in livestock, and attempt to summarize opinions from latest studies in its application.

Yucca schidigera

Yucca schidigera (YS), also named as yucca, is a member of Agavaceae family. The potential of YS has been valued since it was used to treat inflammatory illnesses effectively [20]. Being a tropical plant, YS originally grows in North America, especially in arid Mexican dessert [21]. Beneficial effects of *Yucca schidigera* extracts (YSE) are covering many aspects such as producing desired nutritional attribute that improving feed conversion efficiency thus enhancing animal growth, contributing to environmental control in commercial rearing conditions, and participating in microbial activity modification (e.g., anti-protozoal activity) [21,22].

As a rich source of phytochemicals with promising bioactive functions [23], YS has several components such as steroidal saponins, polyphenolics (e.g., resveratrol and some other stilbenes including yuccaols A, B, C, D and E) [20,24,25]. With 10% of steroidal saponins in its stem dry matter [26], YS has been perceived as one of the two major commercial saponin sources, the other one is *Quillaia saponaria* [21]. Saponins have been considered to be vital components of YS in odor control in intensive farming industry [19,27]. The 3-dimensional spatial orientation, its lipophilic aglycon, and the sugar composition all together contribute to the biological properties of YS [28]. In addition, new steroidal saponins with different structures included in YS have been detected continuously [29-31]. Future analysis of the YS molecule structures, isolation of YS bioactive components, and ascertaining its purity will provide more evidence for YSE application in terms of ameliorating the environmental pollution from livestock industry, and increase the feed efficiency in diets at the same time.

EFFECTS OF *Yucca schidigera* ON GAS MITIGATION IN LIVESTOCK

In Ruminants

Studies of YSE application in gas mitigation area have mostly been focused on ruminants, especially in cattle and sheep (Table 1). Emitting gases were mainly measured in cattle-based experiments. Singer et al. [32] reported that with increased feeding of YSE to lactating dairy cows, 4 h and 24 h gas production generated through these collected rumen fluids were increased, exhibiting a strong linear effect (P<0.05). A similar result was observed in another *in vitro* experiment which involved different ruminal substrates including soluble potato starch, cornstarch, or hay plus concentrate (1.5:1) in the incubation process [33]. Total gas productions at 6 h and 24 h were increased as dietary sarsaponin increased from 1.2 to 3.2 g/L, and the methane reduction rate was statistically up to different substrates. Methane production was decreased (P<0.05) by YSE addition in both gas production rate (mL/min) and extent (L) in the study of Pen et al. [34]. In another research, methane production at 24 h was decreased (P<0.05) by 110 g/kg of YSE addition, although *in vitro* gas production was not affected [27]. Holtshausen et al. [35] indicated that in order to avoid the potential side effects of YSE on ruminal fermentation and feed digestion, saponin levels were reduced (10 g/kg of DM) that resulted in a non-significant difference of methane production *in vitro* among different treatments. However, when sarsaponin concentration was 1% of DM (22.4 g), YSE addition in diets resulted gas reduction in steers effectively, in which methane was inhibited by approximately 12.7% (P<0.05) from day 6 to day 9 of the 10 days feeding period without impairing animal performance [36].

There are also some studies showed inconsistent results. YSE supplementation of 3 g/kg of DM did not reduce methane production in lactating dairy cows, as suggested by Zijderveld et al. [37]. Similar results were also observed by Li and Powers [38] who measured gaseous emissions in room exhaust air of steers. In their study even the 1.5% YSE inclusion groups failed to alter either methane, or ammonia, or nitrous oxide emissions on a daily basis (per unit DMI). Methanol extract of YSE was used in an experiment *in vitro*, and YSE decreased (P<0.05) methane production when calculated by per unit of dry matter, but not by per unit of true digested dry matter [39].

Most researches using sheep as experimental animals were conducted to measure ruminal fermentation parameters related to gas production such as ruminal ammonia concentration. The results of an experiment *in vitro* showed that 100 mg/kg dietary sarsaponin of DM (600 mg/kg CP) reduced the ruminal ammonia over 21% throughout the measurements from day 5 to day 10 [40]. In the subsequent study *in vivo*, only 2 and 30 mg/kg of DM YSE were added in the diets [41]. The results showed that dietary YSE only had slight trends to reduced gas emission without statistical effect over a 15 days period [41]. Feeding the diets with 120 ppm YSE in sheep resulted that YSE reduced N losses in urine and total N losses, leading to a 50% higher retained N, and ammonia N concentration was lowered by 11.9% although not significant [42]. In the subsequent experiment [43], the supplementation of YSE in the basal diet was 240 ppm DM per day and dietary YSE feeding lasted 14 days in which it comprised of 8 days of dietary adjustment. Compared to the control diet, YSE reduced rumen ammonia N concentrations (P<0.05) in Cheviot wethers [43]. A decline of rumen ammonia N was explained that caused by dietary YSE [44].

At 4 h and 6 h after feeding YSE-containing diets (300 mg/kg) in sheep, propionate concentration was increased and acetic concentration was lowered, but neither of them changed significantly [45]. At 2 h after feeding YSE-containing diets (300 mg/kg), protozoan population was decreased (P<0.05). And all 100, 200, and 300 mg/kg YSE feeding resulted a increasing in ammonia concentration [45]. When supplementing 170 mg/d YSE with two other additives (flavomycin and ropadiar) in sheep diets, rumen liquor samples were taken on the day 9 and day 11, and gas production from the sheep was measured from day 12 to day 14. Results indicated an increase in VFA concentration and a decrease in acetate:propionate ratio (P<0.05), while ammonia N concentration (P<0.05) and average methane production (P<0.05) were reduced compared to the control [46]. A later experiment *in vivo* reported that ruminal ammonia concentration, ammonia N concentration and protozoa population in sheep were suppressed especially by the 200 and 300 mg/kg YSE treatment groups in the experimental conditions where dietary YSE levels were 100, 200, 300 mg/kg [47].

For methane production, a recent study revealed that methane production was not affected (P>0.05) by YSE-contained diets, even at highest levels (6 g/d saponins) [48]. Nonetheless, in a latest research, YSE reduced methane significantly (P<0.05) in a dose dependent manner, in both substrates (dates byproducts and the vetch-oat) used in the trials [49]. When saponin levels were over 8 mg/mL, the decreasing percentages of methane can be as high as 60% [49]. Decreased methane production (11%) in wether sheep by YSE (14 mL) in two 23 h runs (day 16 to day 17 of the 18 days period) was noted as well [34].

In Monogastric Animals

This section summarized the studies of two typical monogastric animals: swine and poultry. A number of studies have been carried out to determine the effects of YSE on reducing ammonia in poultry farms. Cabuk et al. [50] reported that feeding of 120 mg/kg dietary YSE resulted in a decreased ammonia concentration of broiler houses at day 19 without impairing broiler performance. However, in another experiment, the supplementation of 100 ppm of YSE and *Quillaja saponaria* was added in a corn-soybean control diet, and ammonia emission of broiler chicken litters was not altered compared with control in the 42 days experimental period [51]. When YSE was applied to laying-hens, 100 ppm inclusion in diets significantly reduced ammonia emission by 44% and 28% for the first two days of manure storage [52]. However, an experiment showed that ammonia N concentrations and microorganism levels of litter materials (half was wood shavings, the other was rice hull) among examined groups did not show statistical difference when pulverized YSE was applied to different litter materials at the level of 0, 4% and 8% [53]. It was hypothesized that the efficiency of YSE could be amplified if litter was used in farming houses under bad situations [53]. As a study to evaluate the effects of YSE on poultry manure alone or together with microbial preparation, YSE showed highest potentials in reducing volatile odorous compounds concentrations after 96 h of the process [19]. This study also confirmed the ability of YSE to decrease the concentrations of odorous compounds emitted from poultry manure such as ammonia, trimethylamine, dimethylamine, isobutyric acid and hydrogen sulfide. In addition, applying YSE separately with microbial preparation at 48 h interval obtained best results [19].

Only few studies have been carried out using swine as experimental animals. Panetta et al. [54] observed no significant effect of dietary YSE (0, 62.5, 125 mg/kg) on ammonia emission during 72 h of consecutive measurement after 4 days dietary adjustment. A decreasing tendency (P>0.05) in ammonia gas production of fecal samples was shown during a 30 days experiment period [55]. However, Liang et al. [56] indicated that YSE added in the feed (125 mg/kg) decreased the emission of ammonia and hydrogen disulfide in the 35 days trials.

Table 1 – Studies oF the effects of YSE on ammonia and methane mitigation in ruminants

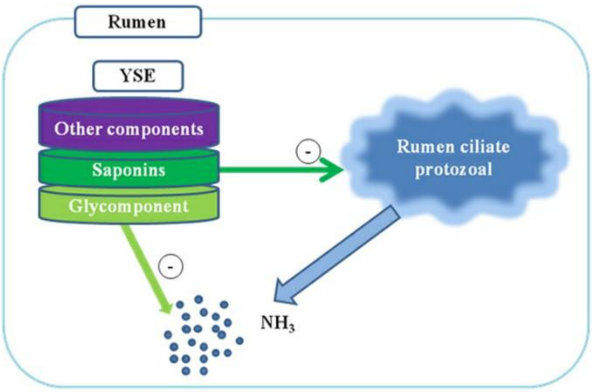
Reference	**Animals**	**YSE Levels**	**Results**
27	Dairy Cows (*in vitro*)	0 or 110 g/kg	CH_4 production at 24 h was reduced (*P*<0.05).
32	Dairy Cows (*in vitro*)	0, 5, 10 or 15 g cow/d (Sarsaponin)	Rumen NH_3-N levels tended (*P*=0.06) to decrease with increased YSE level.
33	Dairy Cows (*in vitro*)	0, 1.2, 1.8, 2.4, 3.2 g/L (Sarsaponin)	Fermentation of soluble potato starch (*P*<0.05) , cornstarch (*P*<0.05), or hay plus concentrate (*P*<0.05) decreased CH_4 production with the concentration of sarsaponin increased.
34	Dairy Cows (*in vitro*)	0, 2, 4, and 6 mL/L (80-100 g/kg saponin)	Rate and extent of CH_4 production were reduced (*P*<0.001) by YSE addition in a dose-dependent manner by up to 42% and up to 32%.
39	Cattle (*in vitro*)	650 µg/ml (100 µg/mL steroidal sapogenin).	YSE treatments decreased CH_4 production when measured as per unit of DM (*P*<0.05).
40	Cow (*in vitro*)	1, 20 and 100 mg/kg DM (sarsaponin)	Substantive effects of the saponin-rich products on ruminal nitrogen metabolism (CH_4, ruminal NH_3 level) were observed only at doses exceeding those recommended by the manufacturers.
35	Dairy Cows (*in vitro* and *in vivo*)	*in vitro*: 15, 30, 45 g/kg DM; *in vivo*: 10 g/kg of DM (6.0% saponin)	Methane production *in vitro* was lowered (*P*<0.05), yet methane *in vivo* was not affected either at low or high supplementations.
36	Steers	0, 11.2, 22.4 g (sarsaponin)	Approximately 12.7% of CH_4 was inhibited (*P*<0.05).
37	Dairy Cows	3 g/kg DM	CH_4 production was not affected.
38	Steers	Experiment 1: 0.64% YSE (8.5% saponin); Experiment 3: 1.5% YSE of DM	CH_4, NH_3, N_2O monitored in room exhaust air were not affected.
49	Sheep (*in vitro*)	0, 2, 4, 6, 8 mg/mL (saponins: 44 g/kg DM)	CH_4 production was reduced (*P*<0.05).
41	Lambs	2 and 30 mg/kg DM (sarsaponin)	CH_4 production was not affected (*P*>0.05).
42	Lambs	120 ppm	NH_3-N was not affected (*P*>0.05).
43	Lambs	240 ppm	Ruminal NH_3 concentration was decreased (*P*<0.05), urinary N was lowered (*P*<0.01).
44	Lambs	14 mL (1.31-1.64 g of saponins /wether/d)	NH_3-N and total VFA concentrations declined (*P*<0.001) with administration YSE.
45	Lambs	100, 200, 300 mg/kg	Ruminal NH_3 concentration was decreased (*P*<0.05).
46	Sheep	170 mg/d	NH_3-N concentrations was lowered (*P*<0.05).

47	Sheep	0, 100, 200, 300 mg/kg	The 200 and 300 mg/kg YSE groups have a suppressing effect on ruminal NH_3 concentration (*P*<0.05) than control.
48	Sheep	0, 1.5, 3.0, 4.5, 6.0 g/d of saponins	CH_4 emissions were not affected (*P*>0.05).

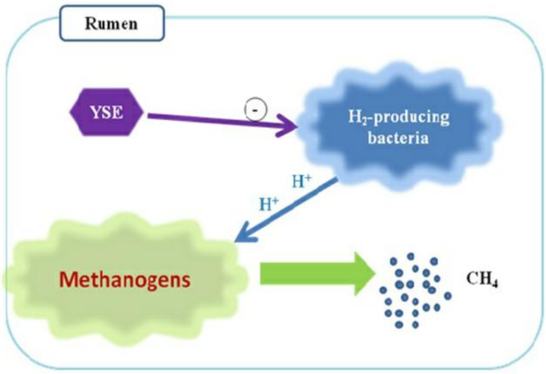
Gas Mitigation Mechanisms

Gas Mitigation Mechanisms of Ruminants

Based on the research of Headon et al. [24], the two components of *Yucca schidigera*, the glycocomponent and the saponin fraction, act differently in binding ammonia in rumen (Fig. 1). The glycocomponent has an ability to bind ammonia directly, while saponin fraction may inhibit ammonia concentrations by membranolytic properties through altering rumen ciliate protozoa, as it (saponin fraction) can cause cell lysis by acting with cholesterol in membranes of protozoal cell [21]. However, the indirect way to reduce ammonia concentration through saponin may contributes most to ammonia reduction since the suppressing potential of glycocomponent is limited [57].



As it is shown by Figure. 2, the most convincing mechanism for methane suppressing effects of steroidal saponins containing plants, YSE specifically, is that methane is possibly reduced through an inhibition of the growth of H_2-producing bacteria [57,58]. It has been demonstrated that ciliate protozoa, which provides substrate (H_2) for methanogens [59,60], is associated with 9-25% of ruminal methanogens [61]. Reduced methane emission due to saponin addition is regarded as the result of its toxicities towards protozoa population [35,62]. The symbiotic relationship between methanogens and protozoa in the ruminal environment accounts at least partially for decrease in methane production due to YSE inclusion in diets [37]. When YSE is added in diets, the balance between methanogens and protozoa would be broken which will lead to methanogens reduction, and eventually influence the production of the emitting methane [36].



Given that the equation (Methane = (1.8 × acetate - 1.1 × propionate + 1.6 × butyrate) / 4) presented by Moss et al. [63], the improvement of propionate production can lead to the reduction of methane production in rumen [34]. This is also supported by Cieslak et al. [64] who reported that the propionate production contents with methane for available hydrogen. *S. ruminantium*, the most predominant bacterium in the process of succinate decarboxylation, accounts for the majority of propionate yield in the rumen [65,66]. Narvaez et al. [39] further noticed that *S. ruminantium* were significantly increased with YSE supplementation, indicating a positive transaction of microbial population towards those propionate-producing bacteria. However, it is possibly that the interaction between protozoa and methanogen has been overestimated [27], as the protozoa viability at specific time in the experiment had not been identified [34]. Goel et al. [67] provided that there was no connection between methanogens, protozoal population and methane production when using different saponin-rich materials such as *Carduus* and *Sesbania* to conduct the study *in vitro*. According to Lila et al. [33], YSE addition can only decrease protozoal populations at 6 h of fermentation in *in vitro* batch cultures since samples collected at 24 h had no detectable protozoa. It seems that YSE has a short lived effect on protozoa *in vitro*, which gives us a partly explanation about reduction of methane with YSE addition.

The pH value is another factor that has an impact on YSE function on the reduction of methane production in rumen. In general, reduction of methane production entails an alteration in total VFA concentration [64] which relates strongly with the acetate/propionate ratio, and this ratio is dependent partly on pH [49]. Cardozo et al. [68] observed that YSE increased the proportion of propionate at pH 5.5, but not at pH 7.0 in *in vitro* rumen environment. CO_2, an end product of lactate fermentation to propionate [69], may contribute to the increasing of total gas production [33]. It is suggested that with the increasing of sarsaponin levels in rumen, CO_2 would be generated through succinate:propionate pathway [33].

Lila et al. [36] pointed out that the lowering of ruminal ammonia concentration in response to YSE to a less extent could be attributed to an inhibited deaminative activity. Gram-positive bacteria and protozoa may be inhibited due to the sarsaponin inclusion which resulted in the falling of ruminal ammonia concentration [33]. Although both experiments *in vitro* and *in vivo* have demonstrated the decreasing of ammonia [58,70,71], the effects could only be observed when at higher application rates of YSE [72]. When at low application rates, YSE processed little biological effects on ruminal ammonia utilization [73]. When high levels of YSE (i.e., > 5 mg/mL) [32] were added in diets, protease activity may be increased, while deaminase activity was unchanged to avoid dietary protein degrade to ammonia [58]. Rumen ammonia N levels tended to reduce with the increasing levels of YSE at high application rates [32,74]. However, it remains a question about the mechanisms of ammonia reduction in response to YSE when short incubation time was incorporated in the experiment [73].

Gas Mitigation Mechanisms of Monogastric animals

According to Liang et al. [56], urease activity might be inhibited efficiently with YSE inclusion, which would decrease the speed of ammonia N formation from urea, so the increasing trends of ammonia N concentrations would be reduced. The dynamic balance of N would be broken in this moderate manner, hence ammonia emission rate would be lowered down. As for hydrogen disulfide reduction, it is hypothesized that YSE may decompose the generation of dissolvable sulfide by inhibiting sulfate reducing bacteria or involves in the process where sulfate reductase participate. It is speculated that antimicrobial abilities of saponins may also accounted for the high efficiency of YSE in dealing with odor from poultry feces [19]. The positive effect of YSE on ammonia reduction may also due to the readily volatilized ability of urinary ammonium which is part of ammonia emitted from manure [54].

Uric acid also has a positive effect on ammonia volatilization [75]. Moisture concentration in manure, which can be changed by YSE [52], is linked to the transformation of decomposition of uric acid directly [76]. But these conversions (solid urea dissolution and urea hydrolysis) need to be finished prior to ammonia emission [77]. Higher pH levels (above 7.0) which can be observed with YSE inclusion [52], is favorable for ammonia release since ammonia is a major form of gas emitted under this condition [78-80]. Factors such as different collection time correspond with varying degrees of manure moisture [52], which need to be noticed to minimize the inaccuracy of experiment as well. Onbasilar et al. [53] attributed the lack of effect in the experiment to exactly relatively low moisture and pH levels.

Possible Reasons of Conflicting Results

Numerous researches have been carried out in an attempt to find the convincing benefits of YSE with regard to gas mitigation, but the results of different studies are contradictory which to some extent were attributed to experimental designs and some non-YSE-structure factors. This part summarized possible factors from the past studies that may have impacts on the accuracy of experimental results, providing basis for the designing of more comparable experiments in the subsequent studies.

Feed Source and Dietary Composition

Feed source and dietary composition can not be ignored when the effects of YSE on gas mitigation are being investigated. An experiment *in vitro* showed that methane production from ruminal fluid was decreased differently with the increasing of sarsaponin concentration when using soluble potato starch, cornstarch, or hay plus concentrate (1.5:1) as substrates, either at 6 h and 24 h [33]. However, Hristov et al. [73] did not observe significant effects of YSE on ruminal fermentation after 8 h incubations *in vitro*. But substituting 50% corn by barley grain in feed mixture (alfalfa hay, grain, soybean meal) had a positive effect on utilization of ammonia N *in vitro* [73], suggesting that changing of specific components in the diets might be beneficial to the digestion of animals, which may make it easier for YSE to show its potential. Singer et al. [32] indicated that 4 h gas production of YSE-modified rumen fluid was affected by different levels of starch in feedstuffs. It is hypothesized that fiber digesting bacteria was reduced with increasing levels of YSE, which would improve amylolytic bacterial population and/or activity, leading to higher yet different gas production among different feedstuffs, thus offered us the notion that the composition of the diet impacted the effects of YSE on gas mitigation.

Xu et al. [27] observed no interaction between YSE dosage either with forage source or the ratio of forage:concentrate in diets. Nonetheless, decreased YSE dosages were accompanied with increasing dietary concentrate values [81-83]. So a relatively high concentrate-contained ration is recommended in order to obtain better effects of YSE on methane reduction. Propionate concentration, which is related to methane production [63], was increased by YSE addition in ruminal fluid *in vitro* [35]. This trend would be more obvious for a high-grain diet than for high-forage diets [74,84,85]. In addition, both methane production [86,87] and ammonia emission [88] in rumen are vulnerable to dietary protein levels. Reduced nitrogen emission might be larger with more pronounced protein deficit (>10%) in diets than diets with adequate protein level [41]. Therefore, maintaining the proper nutritional levels in diets is one of the prerequisites in expressing the potential benefits of YSE with regard to gas control.

Incubation Time (*in vitro*) or Storage Time (Manure)

It is known that fermentation time is essential in fermentation process. Short time fermentation, 4 h in particular, was best chosen for better assessment of gas production, because bacterial population was on the peak value at this time and fermentation parameters would be more reliable [89]. Wang et al. [90] reported that deglycosylation of saponins from YSE occurred at 4 h *in vitro*, which may result in microbial inactivation of the saponins by the formation of sapogenin. While 24 h is mainly for the investigation of feed Metabolic Energy [91]. Singer et al. [32] confirmed the differences of YSE on gas production at different time by monitoring 4 h ($P<0.01$) and 24 h ($P<0.05$) gas production *in vitro*. Rumen microbial adaptation induced by high levels of YSE may be responsible for longer incubation such as 24 h [70]. Wang et al. [92] also confirmed the existence of microbial adaptation of YSE during ruminal fermentation. Therefore in order to make it more significant in terms of gas mitigation at longer incubation time, higher levels of YSE feeding may be more desirable.

When dealing with manure, determining gas emissions from feces at different storage time would lead to various results. In an experiment *in vivo* with 22 days storage time, ammonia emission from room exhaust air in steer house was not affected by YSE supplementation, accounting for only 12% of N losses because 33% of N was retained in manure in the form of ammonia N [38]. This was not corresponding with other studies that an average 44.3% of N losses was through volatilization, most likely as ammonia [93]. Furthermore, during the study period, if the transformation is consecutive, ammonia emissions may be also as a result of organic N to inorganic N [38].

Chemical Composition of YSE Products

Saponins from different sources vary in their contents which would affect bioactivities in rumen fermentation [35,94]. In addition, with different extraction methods [95], the active components of YSE measured using different methods could be inconsistent. For instance, whole-plant product containing polyphenolics may account for some of the bioactivities of YSE [96], which made the effects of YSE on gas mitigation not exclusive to saponins. Furthermore, agronomic and environmental factors like vegetative stage of plant at harvest also influence plant content of YSE [23,28,97]. And some contents of YSE was suspected to show negative effects towards livestock even they possess a GRAS (generally regarded as safe) label [98], and it has not been reported if this is related to extracting methods of YSE.

Different Expressions of YSE Addition Supplied

Although there are numerous reports about YSE application in gas mitigation process, it remains a challenge to make meaningful comparisons among these studies. Levels of YSE in different researches were expressed in various ways such as on the basis of substrate addition (i.e., g/kg of DM) or volume (i.e., g/L) [35], or as actual saponin concentration, or saponin source concentration, but the purity of the saponins used can attribute to various effects [99]. These would make it even hard for effective comparisons.

Different Measurement Methods of YSE Concentration and Gas-Producing-Related Parameters

It is different among saponin determining methods (e.g., Wang et al. [58]: smilagenin equivalents; Holtshausen et al. [35]: butanol-extracted solubles). For methanogen determination, it would be not accurate if it is determined through culture-based techniques, because only part of microorganisms would be cultured due to its non-specificity [100]. But when incorporating a marker (purine) or ^{15}N into the rumen, more integral results of YSE enhancement to microorganisms were obtained [101]. In addition, the determination of ammonia concentration and its emissions was processed in different ways, which is probably one of the reasons for discrepancies in observations [102]. The gap between two methane measurements, chamber measurements and SF6 technique, is over 4% since chamber measurements can also monitor methane emission of rectum besides respired and eructated emission [103].

CONCLUSIONS

This review primarily covered studies of YSE with regard to its application in gas mitigation and summarized some characteristics related to gas mitigation of cattle and sheep in methane reduction, swine and poultry in ammonia reduction. In this article we also summarized possible factors that may affect the results of studies on YSE. Although the results of some studies are conflicting, many articles reported positive effects of YSE on methane/ammonia mitigation, and most of the studies were carried out *in vitro*. Further finely designed *in vivo* experiments of ruminants, especially in cattle, are recommended. Furthermore, the contradictory results of different studies to some extent are attributed to the experimental designs among various studies. So the unification of gas measurement methods and YSE supplementation would make it easier for the comparisons among different studies.

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