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### **Review Article**

## Potentials of using dietary plant secondary metabolites to mitigate nitrous oxide emissions from excreta of cattle: Impacts, mechanisms and perspectives

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#### ABSTRACT

Nitrous oxide (N<sub>2</sub>O) is a potent greenhouse gas as well as the key component depleting the ozone sphere of the earth. Cattle have high feed and water intakes and excrete large amounts of urine and feces. N<sub>2</sub>O can be produced from cattle excreta during storage and use as fertilizer. Mitigating the N<sub>2</sub>O emissions from cattle excreta during production is important for protecting the environment and the sustainable development of the cattle industry. Feeding cattle with low-protein diets increases N utilization rates, decreases N excretion and consequently reduces N<sub>2</sub>O emissions. However, this approach cannot be applied in the long term because of its negative impact on animal performance. Recent studies showed that dietary inclusion of some plant secondary metabolites such as tannins, anthocyanins, glucosinolates n<sub>2</sub>O emissions from cattle excreta. This review summarized the recent developments in the effects of dietary tannins, anthocyanins and glucosinolates on the metabolism of cattle and the N<sub>2</sub>O emissions from cattle excreta and concluded that dietary inclusion of some concluded that dietary inclusion of cattle and the N<sub>2</sub>O emissions from cattle excreta. This review summarized the recent developments in the effects of dietary tannins, anthocyanins and glucosinolates on the metabolism of cattle and the N<sub>2</sub>O emissions from cattle excreta.

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#### 1. Introduction

Global warming has garnered more attention in recent years. It is caused by increasing levels of greenhouse gases, mainly including CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O (IPCC, 2014). Average molar fractions of greenhouse gases in the atmosphere hit high records in 2019, with 410.5  $\mu$ L/L of CO<sub>2</sub>, 1,877  $\mu$ L/L of CH<sub>4</sub>, and 332.0  $\mu$ L/L of N<sub>2</sub>O, respectively (WMO Greenhouse Gas Bulletin, 2020). Although the total amount of N<sub>2</sub>O emissions from the natural environment and human activities is less than CO<sub>2</sub> and CH<sub>4</sub>, the intensity of the greenhouse effect of N<sub>2</sub>O is 265 and 28 times of CO<sub>2</sub> and CH<sub>4</sub>,

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respectively (IPCC, 2014). N<sub>2</sub>O is also a key factor that depletes the ozone sphere of the earth and causes acid rain (Ravishankara et al., 2009). The N<sub>2</sub>O emissions from livestock farming account for 65% of the anthropogenic N<sub>2</sub>O emissions (Steinfeld et al., 2006) and about 75% to 80% of the total agricultural N<sub>2</sub>O emissions (Nasiru et al., 2021). This review will introduce the processes and mechanisms of N<sub>2</sub>O formation in the excreta of animals and summarize the advances of N excretion, as well as the effects of dietary supplementation with plant secondary metabolites (PSM), to decrease the N<sub>2</sub>O emissions from cattle excreta.

#### 2. N<sub>2</sub>O formation in cattle excreta

The N<sub>2</sub>O from cattle excreta is formed through the processes of nitrification and denitrification of nitrogenous compounds which are caused by soil microbes and regulated by soil water-filled pore space (Harris et al., 2021). Fig. 1 shows that urea – which is the major nitrogenous compound in cattle urine – can be degraded into NH<sub>3</sub> by microbial urease in the environment. The majority of NH<sub>3</sub> is transformed into NH<sub>4</sub><sup>+</sup> and any remaining NH<sub>3</sub> evaporates. Consequently, NH<sub>4</sub><sup>+</sup> in excreta and soil is transformed into NO<sub>2</sub><sup>-</sup>,



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**Fig. 1.** Transformation of nitrogenous compounds in soil (modified from Levy-Booth et al., 2014). The *amoA* gene encodes NH<sub>3</sub> monooxygenase; *napA* and *narG* genes encode NO<sub>3</sub> reductases; *nirK* and *nirS* genes encode NO<sub>2</sub> reductases; *norB* gene encodes NO reductase; *nosZ* gene encodes N<sub>2</sub>O reductase; *nxrA* gene encodes NO<sub>2</sub> oxidase; *nifH* gene encodes nitrogenase; DNRA: Dissimilatory nitrate reduction to ammonium.

catalyzed by NH<sub>3</sub> monooxygenase (encoded by *amoA* gene) and hydroxylamine oxidoreductase, and NO<sub>3</sub><sup>-</sup> is catalyzed by NO<sub>2</sub><sup>-</sup> oxidase in the processes of nitrification under aerobic conditions. Then, NO<sub>2</sub><sup>-</sup> is transformed into NO by NO<sub>2</sub><sup>-</sup> reductase (encoded by *nirK* and *nirS* genes) through nitrifier denitrification, and finally reduced into N<sub>2</sub>O by NO reductase through denitrification processes (Levy-Booth et al., 2014).

 $N_2O$  can also be produced as an intermediate product through the process of denitrification under anaerobic or low-oxygen conditions, in which  $NO_3^-$  and  $NO_2^-$  are transformed into  $N_2$  by dissimilatory reduction processes catalyzed by several reductases (Zhu et al., 2013). Fig. 1 indicates that decreasing the total N content is a direct way to reduce the  $N_2O$  emissions from cattle excreta. The N excretion including fecal N and urinary N is positively correlated with the N intake in cattle (Burke et al., 2008). Feeding cattle with low-protein rations would therefore improve the N utilization rate (Castillo et al., 2001) and decrease the total N excretion, especially the urinary N which may reduce the  $N_2O$  emissions.

Fig. 1 also shows that inhibiting the activities of the enzymes which catalyze the processes of nitrification and denitrification could be another approach to decrease  $N_2O$  emissions. Previous studies showed that thiosulfate could decrease  $NH_4^+$  concentrations derived from the degradation of urea by inhibiting soil urease activity (Margon et al., 2015) and reduce the  $N_2O$  emissions from the soil applied with urea (Cai et al., 2018). Dicyandiamide, hippuric acid, and benzoic acid are also well-known nitrification inhibitors that could depress soil nitrification and subsequently attenuate the  $N_2O$  emission of urine patches from ruminants (Bertram et al., 2009; Minet et al., 2018). Thus, dietary manipulation to increase the concentrations of nitrification inhibitors in the excreta of cattle is an important approach to reduce  $N_2O$  emissions.

#### 3. Nitrogen utilization in cattle

Cattle are large ruminants with high feed and water intakes which results in large amounts of feces and urine. The average output of total excreta of beef cattle of 180 to 500 kg in liveweight is 13 to 32 kg/d (Smith and Frost, 2000), and the outputs of feces and urine of fattening cattle of 455 kg in liveweight are 7.5–11.3 kg/d and 7.2–15.5 kg/d, respectively (Li et al., 2014). The N excretion of beef cattle with an average liveweight of 455 kg is about 81.0% to 84.1% of the total N intake (Li et al., 2014). Dong et al. (2014) summarized the N utilization data of beef cattle including 180 mean values of 869 animals in 49 trials and found positive correlations between the N excretion (g/d) and the N intake (g/d): Fecal N excretion = (0.20  $\pm$  0.01) N intake + (15.82  $\pm$  1.88) (n = 180, r = 0.806); Urinary N excretion = (0.51  $\pm$  0.02) N intake -

 $(14.12 \pm 2.71)$  (n = 180; r = 0.878). The results showed that the higher the N intake, the greater the excretion of fecal and urinary N, and subsequently, the greater the NH<sub>3</sub> emission and NH<sub>4</sub><sup>+</sup> formation during excreta storage (Todd et al., 2013). Large amounts of excreta and low N conversion rates not only waste protein from feedstuffs and increase feed cost, but also increase the precursors for N<sub>2</sub>O formation. Therefore, reducing the dietary N intake of beef cattle is an important option to decrease N excretion and consequently N<sub>2</sub>O emissions from cattle excreta. However, feeding cattle with lower dietary N levels than required over a long period is unacceptable in animal production. Investigating the options to inhibit the activities of enzymes related to nitrification and denitrification for N<sub>2</sub>O formation and thereby mitigate the N<sub>2</sub>O emission would be more important.

# 4. Impacts of urinary nitrogenous compounds of cattle on $N_2 O \mbox{ formation }$

The nitrogenous compounds in the urine of ruminants mainly include urea, uric acid, creatinine, allantoin and hippuric acid. Urea is synthesized in the liver of cattle using blood NH<sub>3</sub> as the precursor absorbed from the rumen and the hindgut (Bach et al., 2005). Most of the urea can enter the rumen through saliva secretion and via the rumen epithelium, and part of the urea can be excreted into the urine. Table 1 shows the N proportion of different nitrogenous compounds in the urine of beef cattle from different studies. Urea is the main nitrogenous compound and its N proportion of total urinary N averaged 60% to 70%. Urea can be degraded rapidly by microbial urease within soil into  $\mathrm{NH_4}^+$  within a few hours and subsequently transformed into NO2<sup>-</sup> and NO3<sup>-</sup> through the nitrification process in soil (Whitehead et al., 1989). Previous studies reported that urease inhibitors could effectively reduce the degradation of urea in soil and decrease NH3 and N2O emissions from cattle urine (Singh et al., 2013; Zaman et al., 2013). The results suggest that urea in the urine of cattle is the most direct and important N source for urinary N<sub>2</sub>O formation.

Hippuric acid is an arylglycine synthesized in the liver of cattle using benzoic acid and glycine as the precursors and can be excreted into urine. The proportion of hippuric acid-N in the urine N averaged at 2.2% to 7.3% in beef cattle (Table 1). Several studies indicate that hippuric acid is a nitrification inhibitor that inhibits the activities of soil nitrifiers and consequently reduces the N<sub>2</sub>O emissions from cattle urine. Van Groenigen et al. (2006) reported that increasing hippuric acid concentration from 0.4 to 5.6 mmol/kg in artificial urine decreased the N<sub>2</sub>O emissions by 54% during a 64-d incubation of soil. Bertram et al. (2009) reported that increasing the proportion of hippuric acid-N in

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	Item	Da Silva Cardoso et al. (2019)	Gao et al. (2022)	Bao et al. (2018)	Zhou et al. (2019)	Zhao et al. (2021)	Xie et al. (2021)
	Total N, g/L	15.3 (6.7 to 26.9)	2.6 (2.4 to 2.9)	5.1 (4.0 to 6.5)	4.3 (2.5 to 5.5)	6.2 (5.8 to 6.9)	ND
N proportion, % UN							
	Urea	60.4 (43.0 to 70.4)	65.4 (59.2 to 70.3)	70.4 (63.9 to 76.8)	66.0 (57.4 to 77.4)	69.2 (67.2 to 71.9)	64.7 (59.0 to 70.1)
	Allantoin	3.1 (1.9 to 4.5)	6.0 (4.6 to 7.4)	6.9 (5.5 to 8.1)	9.5 (5.7 to 11.2)	9.6 (7.9 to 10.5)	6.1 (5.7 to 6.6)
	Uric acid	ND	0.9 (0.8 to 1.0)	1.1 (0.8 to 1.3)	1.4 (1.0 to 1.8)	0.9 (0.9 to 1.0)	0.2 (0.2 to 0.3)
	Creatinine	3.4 (2.4 to 4.9)	10.5 (10.3 to 10.7)	6.5 (4.3 to 9.2)	9.6 (5.7 to 14.7)	9.4 (8.8 to 10.0)	5.4 (5.0 to 5.8)
	Hippuric acid	5.0 (2.2 to 7.3)	2.7 (2.5 to 2.9)	4.2 (3.2 to 5.8)	4.2 (2.2 to 5.6)	5.1 (4.8 to 5.4)	3.2 (3.1 to 3.2)

ND = not determined or not reported; UN = urinary N.

<sup>1</sup> Data are expressed as mean values (range within brackets). Data in Da Silva Cardoso et al. (2019) contained 5 treatment means with n = 5 per treatment; Gao et al. (2022) contained 4 treatment means with n = 4 per treatment; Zhou et al. (2019) contained 4 treatment means with n = 4 per treatment; Zhou et al. (2021) contained 3 treatment means with n = 6 per treatment; Xie et al. (2021) contained 4 treatment means with n = 8 per treatment; Bao et al. (2021) contained 3 treatment means with n = 6 per treatment; Xie et al. (2021) contained 4 treatment means with n = 8 per treatment.

urinary N from 6.4% to 12.6% decreased  $N_2O$  emissions of artificial urine by 65%. The results indicated the inhibitive effects of hippuric acid on  $N_2O$  formation, and increasing the urinary excretion of hippuric acid could be an option to reduce  $N_2O$  emissions of cattle urine.

Creatinine is the metabolite of muscle catabolism and is directly related to muscle mass in beef cattle (Hayden et al., 1992). The proportion of creatinine-N in urine N varies greatly from 2.4% to 14.7% (Table 1). Urinary allantoin and uric acid are derived from the nucleic acids of the rumen microbes and the total amount of urinary uric acid and allantoin is used as the indicator to predict the ruminal microbial N yield (Chen and Gomes, 1992). The proportion of uric acid-N in urine N is less than 1.8% (Table 1) because uric acid can be oxidized into allantoin in the liver (Tas and Susenbeth, 2007), and the proportion of allantoin-N in urine N is 1.9% to 11.2% in beef cattle (Table 1). No recent studies are available on the positive effects of creatinine, allantoin and uric acid on the N<sub>2</sub>O emissions of cattle urine (Gardiner et al., 2018a).

#### 5. Plant secondary metabolites and their functions

The PSM differ from the primary plant structural components such as carbohydrates, lipids and protein. Kossel (1891) first defined PSM as opposed to plant primary structural components and demonstrated that PSM play important roles in plants to adapt to the environment. The PSM are characterized by low abundance, often below 1% of the total carbon in plants, and their storage usually occurs in dedicated cells or organs of plants (Bourgaud et al., 2001). Based on their biosynthetic pathways, PSM are usually divided into 3 large molecule families including phenolics (tannins, anthocyanins, proanthocyanidins, etc.), terpenes and steroids (monoterpenes in essential oils, aucubin, saponins, phytosterol, etc.), and alkaloids (theophylline, berberine, theine, etc.) (Harborne, 1999). Most PSM have the ability to protect the plants from damage or disoperation by animals or microorganisms. Kessler and Kalske (2018) summarized that PSM protect the plants from ingestion by herbivores through the direct defenses by toxic and antinutritional PSM and the indirect defenses of PSM to cause poor palatability of plants for animals. In the last decade, more attention has been paid to the inhibitive effects of PSM on the ruminal CH<sub>4</sub> emissions, such as saponins (Mao et al., 2010), tannins (Jayanegara et al., 2012) and essential oils (Castro-Montoya et al., 2015). In recent years, it is reported that PSM released from the roots of some plants had inhibitive effect on the nitrification processes in soil (Subbarao et al., 2009). Some PSM included in feeds could be hydrolyzed in the gut of animals and their metabolites could be excreted into urine and consequently reduce urine N2O emissions (Sanchez-Martín et al., 2017; Bao et al., 2018).

## 6. Effects of dietary plant secondary metabolites on mitigating urine $N_2O$ emissions

#### 6.1. Tannins

6.1.1. Hydrolysable tannins and their metabolites

Tannins are polyphenolic compounds in plants and could be divided into hydrolysable tannins and condensed tannins (CT) (Fig. 2A, McSweeney et al., 2001). Tannic acid is a type of hydrolysable tannin composed of 8 to 10 molecules of gallic acid. Yang et al. (2016) reported that dietary addition with tannic acid at 26.0 g/kg dry matter (DM) decreased the urinary N excretion and increased hippuric acid excretion in beef cattle. Zhou et al. (2019) reported that dietary addition with tannic acid at 2 dietary crude protein levels (11.1% vs. 13.6%) increased the ratio of hippuric acid-N to urinary N in beef cattle and decreased the urine N<sub>2</sub>O–N emissions by 40.7% and 45.8%, respectively.

Effects of dietary addition with tannic acid on decreasing the N<sub>2</sub>O emissions could result from 3 origins. Firstly, tannic acid reduces N<sub>2</sub>O emissions through shifting N excretion from urine to feces because the N<sub>2</sub>O emission factor of fecal N is much lower than that of urinary N (Luo and Kelliher, 2010). Secondly, dietary inclusion with tannic acid increases the urinary excretion of hippuric acid which could inhibit the nitrification process in soil (Kool et al., 2006). Thirdly, tannic acid can be hydrolyzed in the rumen (Fig. 3) (Singh et al., 2001) and the metabolites of tannic acid such as pyrogallol and resorcinol excreted into the urine of cattle inhibit the urine N<sub>2</sub>O formation (Bao, 2019, unpublished results).

Previous studies showed that dietary additions with tannic acid and gallic acid both increased the urinary excretions of pyrogallol and resorcinol in beef cattle (Zhou et al., 2019; Bao et al., 2018). Although gallic acid is the basic structure of tannic acid, dietary addition with tannic acid increased urinary excretions of hippuric acid, whereas gallic acid did not in beef cattle (Bao et al., 2018; Wei et al., 2016). Bao et al. (2018) reported that dietary addition with gallic acid at 15.2 g/kg DM reduced the estimated urine N<sub>2</sub>O–N emission and the N<sub>2</sub>O–N/urine-N application ratio in steers. The results suggest that the metabolites of gallic acid including pyrogallol and resorcinol could have inhibitive effects on decreasing the urine N<sub>2</sub>O–N emissions in beef cattle. Further in vitro research indicated that resorcinol decreased urine N<sub>2</sub>O formation, whereas pyrogallol did not have the impact (Bao, 2019, unpublished results).

#### 6.1.2. Condensed tannins

CT are polyphenolic compounds and cannot be easily degraded in the rumen because the phenolic hydroxyl groups of CT are combined with other macromolecules (Naumann et al., 2013). CT are usually believed to be anti-nutritional factors in feeds, because these compounds depress the feed intake and growth performance



Fig. 2. The basic molecular structure of tannins (A, McSweeney et al., 2001), glucosinolates (B, Possenti et al., 2017), anthocyanins (C, Riaz et al., 2016) and aucubin (D, Zeng et al., 2020). R represents the R chain.



Fig. 3. The degradation of tannic acid in the rumen (modified from McSweeney et al., 2001).

of beef cattle (Reed, 1995). In recent years, many studies showed that dietary addition with CT shifted N excretions from urine to feces in beef cattle. Ebert et al. (2017) reported that dietary additions with quebracho extract containing 95% CT at 0.0%, 0.5%, and 1.0% in rations linearly increased the fecal N excretions and decreased the urinary N excretions without affecting the N intake and the N retention in beef cattle. Norris et al. (2020) who included quebracho extract containing 78% CT at 0.0%, 1.5%, 3.0%, and 4.5% to beef cattle rations and Koenig and Beauchemin (2018) who added the extract from *Acacia mearnsii* containing 53.2% CT at 2.5% to beef cattle rations found similar results as that of Ebert et al. (2017). These results suggest that dietary addition with CT is an effective

way to decrease the  $N_2O$  emissions from beef cattle excreta, because the  $N_2O$  emissions from fecal N is much lower than that of urinary N (Luo and Kelliher, 2010).

#### 6.2. Glucosinolates

Glucosinolates (GLS) are a special type of secondary metabolites which exist only in cruciferous plants. The GLS are water-soluble organic anions with a similar basic structure (Fig. 2B). Based on the side chains, GLS can be classified into aliphatic, aromatic, and indole types. Brassica is an important genus of the Brassicaceae family. Increasing evidence shows that consumption of Brassica vegetables has some beneficial effects for human health, such as decreasing the risk of breast cancer (Liu and Lv, 2013; Zhang et al., 2018). The contents and types of GLS vary widely depending on the species of Brassica plants (Table 2). Balvert et al. (2018) reported that mixing Brassica plant tissues such as kale and turnip with urea decreased N<sub>2</sub>O emissions derived from urea in soil and the N<sub>2</sub>O emission factors compared with ryegrass tissues. Because GLS are the specific secondary metabolites in Brassica plants (Tripathi and Mishra, 2007), it could be speculated that the N<sub>2</sub>O formation could have been inhibited by GLS and its metabolites.

The GLS and myrosinase exist separately within intact Brassica plants. When the plants are masticated and ingested by animals, the GLS can be hydrolyzed by the myrosinase in Brassica plants or microbial enzymes in the digestive tract of animals into several bioactive products such as isothiocyanates (ITC), oxazolidine-2thione (OZT), and thiocyanates (SCN). Previous studies showed that SCN was found in ruminal fluid as well as in the urine of beef cattle fed rapeseed meals (Subuh et al., 1995; Gao et al., 2021a), but ITC and OZT were undetectable in the fluids. The results indicated that the main metabolite of GLS in the rumen was SCN, suggesting that the hydrolyzing pathway in the rumen was different from that activated by myrosinase.

It was reported that some metabolites of GLS were harmful to animals and microorganisms depending on the doses (Baskar et al., 2016; Popova et al., 2017). Bending and Lincoln (2000) showed in an incubation trial that ITC degraded from GLS inhibited the first step of nitrification (NH<sub>4</sub><sup>+</sup> oxidation) both in sandy soil and clay soil through inhibiting the activities of nitrifying bacteria compared with intact GLS and nitriles. Snyder et al. (2010) reported that 2propenyl ITC and SCN degraded from GLS in Brassica juncea and Sinapis alba seed meals inhibited the microbial respiration, and SCN was responsible for the inhibitive effects on nitrification in soil. Balvert et al. (2017) reported that some GL hydrolysis products of GLS decreased the N<sub>2</sub>O emissions from silt loam soil applied with urea (600 mg N/kg soil), and found that phenethyl ITC reduced the N<sub>2</sub>O emissions from the soil by up to 51% in static incubation. These results indicated that the metabolites of GLS in Brassica plants have potential inhibitive effects on the nitrification processes caused by soil microbes and on decreasing urine N<sub>2</sub>O emissions, if the metabolites are excreted in the urine of animals. However, Hoogendoorn et al. (2016) reported that the urine N<sub>2</sub>O emission factors of sheep fed forage rape containing GLS was higher than that of the sheep fed ryegrass. Gao et al. (2022) reported that dietary inclusion with rapeseed cake containing high GLS increased the estimated urine N<sub>2</sub>O-N emissions and N<sub>2</sub>O emission factors in

Table 2					
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Contents and types of glucosinolates in Brassica plants

steers, and the urine N<sub>2</sub>O–N emissions were positively correlated with the urinary SCN excretions. Further static incubation also showed that SCN linearly increased the N<sub>2</sub>O–N fluxes and N<sub>2</sub>O–N/N application ratio of artificial urine (Gao et al., 2021b). The results suggest that feeding cattle with rapeseed cake containing GLS is not beneficial in reducing urine N<sub>2</sub>O emissions. The discrepancy in the results among different studies are unclear which needs to be clarified in further research.

#### 6.3. Anthocyanins and aucubin

Anthocyanins (ATH) are water-soluble pigments and wildly exist in plants. Chemically, ATH are flavonoids mainly composed of aglycones (anthocyanidins) and sugars (Fig. 2C). More than 700 types of ATH are identified which are mainly derived from 6 different aglycones including cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin (Kähkönen and Heinonen, 2003). The ATH were found to have high antioxidative effects in studies with rodents (Sankhari et al., 2012) and goats (Tian et al., 2019). In humans, it was found that consumption of Vaccinium myrtillus rich in ATH increased the serum hippuric acid concentration (De Mello et al., 2017) and oral intake of <sup>13</sup>C-enrichedcyanidin-3-glucoside (a type of ATH) increased the urinary hippuric acid excretion (Czank et al., 2013; De Ferrars et al., 2014). Therefore, it could be speculated that dietary inclusion with some extracts rich in ATH could possibly increase urinary hippuric acid excretion and consequently decrease urine N<sub>2</sub>O emissions in cattle. Gao et al. (2021c) reported that addition with 114 g red cabbage extract rich in ATH per day in the ration of beef bulls increased the hippuric acid-N to urinary N ratio from 6.56% to 7.70% and decreased the estimated urine N<sub>2</sub>O-N emissions by 33.1% through inhibiting the nitrification processes in soil applied with the urine samples during static incubation. This study indicates that oral intake of ATH would be an effective approach to increase the urinary hippuric acid excretion and decrease urine N<sub>2</sub>O-N emissions in beef bulls. However, whether feeding cattle with other sources of ATH such as purple corn (Zea mays L.), barley (Hordeum vulgare L.), and red sorghums have the same effects, needs to be investigated in the future.

Aucubin, an iridoid glycoside (Fig. 2D), is one of the PSM existing in many plants such as *Aucuba japonica* and Plantago plants (Zeng et al., 2020). Both field and laboratory experiments showed that direct addition with aucubin in the urine of cattle decreased urine  $N_2O-N$  emissions (Gardiner et al., 2018b, 2020). Another study showed that feeding cows with 45% DM of *Plantago lanceolata* 

Glucosinolates	Broccoli sprouts <sup>1</sup>	Brussels Sprouts <sup>1</sup>	Cauliflower <sup>1</sup>	Red cabbage <sup>2</sup>	Kale rosette leaves <sup>3</sup>
Total, µmol/100 g fresh weight	402	940	322	462	880
Individual molar proportion, %					
Glucoiberin	14.90	6.24	12.92	8.01	5.57
Glucoraphanin	33.08	1.61	1.41	32.03	1.02
Progoitrin	2.64	14.15	2.66	19.85	0.11
Gluconapin	0.61	11.49	0.42	7.64	58.41
Sinigrin	0.93	16.49	13.04	8.74	8.18
Glucoalysin	0.03	1.17	1.45	0.00	0.00
Glucoerucin	25.37	0.00	0.80	1.86	0.45
Glucobrassicin	0.99	39.79	47.52	0.00	18.75
Neoglucobrassicin	7.76	0.00	7.27	0.00	4.77
4-Methoxyglucobrassicin	13.78	8.98	12.55	10.41	1.14
Gluconasturtiin	0.00	0.00	0.00	2.84	1.25
4-Hydroxyglucobrassicin	0.00	0.00	0.00	1.86	1.14

<sup>1</sup> Tian et al. (2005).

<sup>2</sup> Volden et al. (2008).

<sup>3</sup> Sun et al. (2011).

containing aucubin did not affect urine N<sub>2</sub>O emission factors, but decreased N<sub>2</sub>O emissions through reducing urinary N concentrations (Simon et al., 2019). However, the metabolism of aucubin in ruminants is unclear. Whether aucubin could be absorbed and excreted into urine and further inhibit N<sub>2</sub>O–N emissions needs to be investigated in the future.

#### 7. Perspectives

Present results indicate that dietary inclusion of PSM such as tannins and tannic acid can effectively decrease N<sub>2</sub>O emissions from beef cattle excreta through shifting N excretion from urine to feces. Meanwhile, dietary inclusion of gallic acid or ATH can decrease urine N<sub>2</sub>O emissions through the inhibitive effects of the metabolites of these PSM excreted in the urine of beef cattle. The effects of dietary inclusion of Brassica plants or by-products rich in GLS on urine N<sub>2</sub>O emissions are not conclusive and need to be clarified in further research. Mitigating N<sub>2</sub>O emissions from cattle excreta through the metabolites of PSM has great potential and more research in this field is required. It should be noted that PSM such as nitrates, hydrogen cyanide, and some types of alkaloids may have toxic effects on animal health. Some types of PSM such as phytic acid and OZT degraded from GLS may have antinutritional effects on animals. Additionally, pure PSM extracts are relatively expensive. Plants containing PSM that do not have negative effects on health and nutrient utilization yet effectively reduce N2O emissions are recommended for feeding cattle.

#### Author contributions

**Jian Gao:** Visualization; Writing-original draft preparation; **Guangyong Zhao:** Writing-review & editing; Supervision.

#### **Declaration of competing interest**

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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