Abstract—Livestock, particularly ruminant animals produce methane which plays an important role in global warming and in the destruction of ozone layer. Furthermore, methane production is also associated with considerable energy losses from ruminant and lead to decreasing energy gain and productivity. In this paper, a review is presented of nutritional approaches for mitigating methane production from livestock. The objective can be achieved by ration manipulation, the use of additives or supplements, and biotechnological interventions. Increasing proportion of concentrate and decreasing proportion of roughage in the diet may reduce methane production. Some additives that can be used are lipids, antibiotics and plant extracts. The use of leaves from tannins and saponin-containing plants are also promising options. Some biotechnological interventions are adding probiotics, defaunation, and introduction of reductive acetogenesis in the rumen. However, some of these options often cause detrimental effects to the productivity of livestock and its environment. Therefore, methanogenesis and its inhibition can not be considered as a separate part of rumen fermentation, but rather to the livestock production as an entire system. Moreover, reasonable options and cost efffectivity should be taken into account when applying these methods in the developing countries such as Indonesia.

I. INTRODUCTION

Livestock, both monogastrics and ruminants play an important role as food sources for human, particularly as protein source. They derive various kinds of valuable products such as meat, milk, egg and many others. Therefore to maintain and to increase their production, both in quantitative and qualitative terms is a necessity.

However, instead of their contribution to human, ruminants produce considerable amount of methane which plays an important role in global warming and in the destruction of ozone layer. Ruminant methane production is responsible for approximately 95% of total global animal and human methane emissions [1], and contributes to the anthropogenic greenhouse gases to be as high as 18% [8]. Furthermore, methane production is also associated with considerable energy losses and lead to decreasing energy gain and productivity. Around 6-10% of the gross energy of the ruminant diet is converted to methane [2].

II. METHANE PRODUCTION IN THE RUMEN

The rumen ecosystem is anaerobic. Therefore the oxidations of feed substances such as carbohydrates and protein for energy production are made through dehydrogenations. During oxidation, NAD is reduced to NADH. NADH has to be re-oxidized to NAD to complete the fermentation of sugars:

\[
2H + NAD^+ \leftrightarrow \text{NADHH}^+
\]

\[
\text{NADHH}^+ \leftrightarrow NAD^+ + H_2
\]

The hydrogen gas (H\textsubscript{2}) formed has to be eliminated to maintain the hydrogenase activity and to avoid negative feedback on microbial organic matter degradation. The reduction of CO\textsubscript{2} with H\textsubscript{2} via methanogenesis keeps the partial pressure of hydrogen very low, and this has an important effect on the overall fermentation: hydrogenase activity can proceed towards hydrogen production, thus avoiding the formation of lactate or ethanol as major end products and allowing more acetate to be produced [1]. The removal of H\textsubscript{2} can be through methanogenesis and acetogenesis in the following pathways:

\[
\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \quad \text{methanogenesis}
\]

\[
2\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2\text{O} \quad \text{acetogenesis}
\]

Therefore the formation of CH\textsubscript{4} is an essential metabolic pathway for H\textsubscript{2} removal in the rumen. However, methanogenesis must be optimized for digestive efficiency and can be reduced to a maximum of 10-15% without any detrimental effect on the major rumen functions [3].

III. INHIBITION OF RUMINAL METHANOGENESIS

Inhibition of rumen methanogenesis is aimed mainly at increasing feed efficiency and lowering greenhouse gas emissions. By shifting electron flow from methane to propionate production, more energy and carbon are deposited in short chain fatty acids (SCFA), which become available to the animal [1]. Some of the promising options to mitigate methane production are described in more detail in the following section.

A. Ration Manipulation

The type of carbohydrate fermented influences methane production most likely through impacts on ruminal pH and the microbial population. High amounts of concentrates decrease rumen pH and it is known that methanogenic bacteria are inhibited at lower pH values [1]. Fermentation of cell wall fibre produces higher acetic:propionic acid and higher methane losses [5]. Since roughage contains more fibre and...
concentrate contains more soluble substances, therefore the replacement of roughage in animal diet by concentrate shifts the composition of partial SCFA from higher to lower acetate production and more propionate. In the developing countries this option is still very much open. But in the developed countries, most of ruminant particularly dairy cows are already fed at high concentrate level.

However, the effect of concentrate is not linear, and only very high dietary concentrate proportions seem to be really effective which is why the IPCC (Intergovernmental Panel on Climate Change) set the borderline at >90% concentrate, a level differentiating diets suitable for ruminants from diets used in feedlots [8]. The scope of using concentrates to lower CH4 emissions from the dairy sector is also limited as milk quality is negatively affected once concentrates exceed ~50% of the diet. Furthermore, increased dietary concentrate may sometimes increase total net emissions as more grain must be grown, processed and transported, leading to increased use of pesticides, fertilisers, and additional ancillary sources of emissions associated with production and transportation infrastructure [4]. Forage processing such as chopping, grinding and pelleting can further decrease methane production. Increase rate of passage of the processed forage likely contributes to the reduced methane production [6].

B. The Use of Additives

1) Lipids: Lipids and lipid-rich feeds are among the most promising options for direct methane mitigation. Lipids can serve as a substitute for a part of dietary carbohydrates (maximum of 10% lipids in diet, but actually 2%), and lipids have a negative effect on ruminal methane production. Dietary lipid supplementation reduces CH4 emissions by decreasing ruminal organic matter fermentation, the activity of methanogens and protozoal numbers, and for lipids rich in unsaturated fatty acids, through hydrogenation of fatty acids [5]. Saturated medium chain fatty acids, C10-C14, also contribute to methane reduction. At ruminal temperature, an increasing chain length of medium chain fatty acids seems to reduce their efficiency in inhibiting methanogens and methane formation due to lower solubility [11]. In practice, reductions of 10-25% of methane are more likely although reductions ≥40% are possible with high levels of lipid supplementation. Generally, it is recommended that total fat should not exceed 6-7% of the dietary dry matter otherwise a depression in dry matter intake may occur [4].

2) Antibiotics: For many years, antibacterial substances have been used widely in animal production as growth promoting substances. An antibiotic chlortetracycline (11 ppm in feed) was found to lowering 9-22% methane production than control animals in in vitro incubations. The inhibition was not the result of direct effect of the antibiotic on methanogenic bacteria, but due to inhibition of the microbes producing hydrogen and formate, both intermediate precursors of methane. Other antibiotic avoparcin, a glycopeptide, is known to act on Gram-positive bacteria, inhibit methanogenesis, simultaneously shifting the SCFA pattern to a higher propionate production at the expense of acetate and butyrate. Bacitracin, a polypeptide antibiotic which also affects Gram-positive bacteria, also lowered methane production, but its action was less potent than ionophores [5].

Ionophore additions to beef cattle diets, particularly monensin, reduces feed intake 5 to 6%, decreases acetic:propionic acid and decreases methane losses. The decrease in methane production ranges from slight to approximately 25%. The effect of ionophores is probably due to shifts in the microbial population towards ionophore-resistant organisms, which tend to produce more propionate [5].

3) Plant extracts: As a general trend, European research has concentrated on natural feed additives after the ban of feed antibiotics. Outside of Europe the most promoted feed additive to mitigate methane formation from ruminants still is monensin although there are several constraints including the uncertainty of its long-term effect. Promising natural feed additives include plant extracts, especially those rich in either saponins or tannins [12]. The CH4-suppressing effect of plants rich in saponins seems to be particularly related to their anti-protozoal effects. Saponins form complex with sterols in protozoal cell membranes causing inhibition of their activity and cell lysis [16]. Since a small portion of methane production is due to methanogens attached to protozoa, the decrease of protozoal counts due to the presence of saponins affecting also methane production. Several in vivo and in vitro studies provide evidence for CH4 suppression from some saponin sources such as saponin-rich extracts of Quillaja saponaria and Yucca schidigera [13], although it seems that not all saponin sources are effective [4].

Tannins in the form of hydrolysable tannins have been proved to decrease ruminal methane production in vitro, and the reduction of methane linearly increased by the increase of tannin activity with $r^2=0.99$ and $P<0.001$ using three different tannin containing plants. Tannin activity was expressed as the percentage of gas increase after adding polyethylene glycol (PEG) since PEG has a specific binding to tannins [9]. The authors then extended the sample size using 16 different plants and still got high correlation with $r^2=0.87$ and $P<0.001$ (Figure 1). However, there was no significant relationship between condensed tannins and methane reduction potential [10]. The proposed mechanisms whereby tannins reduce methane emissions from ruminants are: (1) indirectly through a reduction in fiber digestion, which decreases H2 production, and (2) directly through an inhibition of the growth of methanogens [15].
However, intensive research is still required to classify the multitude of chemical forms of saponins and tannins for their efficiency and to evaluate the ideal doses for maximum effects at minimal adverse side-effects. Using saponins properly is also relevant to animal health due to their potential haemolytic activity, while with using tannins the major problem consists of low palatability and potential impairment of ruminal digestion. In turn, both groups of plant secondary metabolites may have co-benefit in reducing ruminal protein degradation to ammonia thus reducing the inclination of the manure to emit environmentally hazardous ammonia [8].

C. Biotechnological Interventions

The opportunities for lowering methane formation in the rumen through microbial intervention are described in this session (Figure 2). One option is specifically to target methanogens by the use of antibiotics, bacteriocins, or phage. Another is to decrease $H_2$ so that less $H_2$ available for methane formation. This can be achieved by removing protozoa from the rumen or by feeding ruminants less fibrous materials and more digestible energy sources such as grains. A third option is to develop alternative $H_2$ sinks in order to divert $H_2$ away from methanogens [7].

Fig. 1 Relationship of tannin activity and percentage of methane reduction

Fig. 2 Possible microbial-intervention sites for lowering ruminant methane

1) Probiotics: The most common used probiotics for ruminants are based on Saccharomyces cerevisiae and Aspergillus oryzae. The addition of probiotics could reduce the number of protozoa. Because the close association between methanogens and ciliates, and between ciliates and other members of the bacterial population, the microbial additives could therefore change the composition of the microbial flora and decrease methane production indirectly by altering the balance of the population [1]. However, the available data relating microbial additives and methane production are not convincing until now and much more research is needed before it can be concluded that probiotics decrease methane production in vivo. For example, Saccharomyces cerevisiae fed to bulls at 1.5 kg per tonne of feed and measured methane production from rumen fluid in vitro, did not decrease methane production from the control after 24 h although there was a 10% decrease after 12 h incubation [14].

2) Defaunation: Ruminal protozoa play an important role in methane production, particularly when cattle are fed high-concentrate diets. Ruminal methanogens have been observed attached to protozoal species suggesting possible interspecies hydrogen transfer [17]. These protozoa-associated methanogens have been variously reported as contributing up to 37% of total rumen methane emissions [19]. Therefore removal of protozoa from the rumen (defaunation) is associated with the decrease in methane production. This was confirmed by a report that protozoa-free lambs produced 26% less methane per kg dry matter intake than faunated lambs, while the proportions of methanogens in total bacterial populations were lower in protozoa-free animals in whole ruminal contents [18].

3) Reductive acetogenesis: Redirection of reducing equivalents from methanogens to acetogens may be another way of decreasing ruminal methane production [18]. Reductive acetogenesis is a natural process which converts $H_2$ into acetate and is a highly desirable alternative to methanogenesis because it yields increased acetate, an energy source for ruminants. Bacteria capable of converting carbon dioxide and hydrogen gas into acetate rather than methane have been isolated from most anaerobic environments including the intestines of insects and humans, and the rumen sheep and cattle [20]. If reductive acetogenesis can be established in the rumen, it offers increased animal performance since it provides a useful end-product for the host animal to metabolise as an energy source as well as decreased greenhouse gas production [7].

IV. SELECTING THE APPROPRIATE METHODS

There are numerous strategies to mitigate methane emissions from livestock and some of the methods are described as above. The question that may arise is, which method is the most appropriate to be chosen? Actually, there is no single approach can completely solve the problem. Each method has its own advantages and disadvantages. Increasing proportion of concentrate instead of roughage will enhance livestock productivity at the same time but too costly for ruminant feed. Chopping the forage is a simple technique and affordable in many developing countries, but require more labor force. Lipid is an interesting option but animals will reject the diet if it is too high proportion of lipids due to its inconvenient smell. Antibiotics and ionophores are very effective in reducing methane but banning of such products is of important to be considered. Tannins and saponins are
abundantly available from nature but may cause adverse effects on palatability and animal health as well. Biotechnological interventions are promising but more research needs to be carried out to establish such technology in practice. In the developing countries such as Indonesia, cheaper and easier technologies are preferable such as chopping, adding lipids to certain extent, and mixing a portion of tannins- and saponins-rich plant leaves to animal diets. Another thing that needs to be considered in selecting the appropriate methods to reduce methane emissions is its effect on the livestock as an entire system rather than only the rumen fermentation. It is unwise to reduce methane production but at the same time creating negative effects on livestock productivity and health, whereby they products are needed to feed to many people in many areas of the world.

V. CONCLUSIONS

The development of nutritional strategies to mitigate methane emissions from ruminant animals is possible and desirable. Mitigating methane emissions is not only improving feed efficiency and animal productivity, but also reducing the contribution of ruminant livestock to the global methane inventory. Some of available methods are based on ration manipulation, the use of additives and through biotechnological interventions. However, many factors need to be considered before applying a particular method, especially its advantages and disadvantages and its relationship with local condition. Reasonable options and cost effectiveness should be taken into account when applying these methods in the developing countries such as Indonesia.

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REFERENCES