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Methane mitigation by using chicken egg yolk (IGY) antibodies generated against methanogens – a review

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ABSTRACT

Methane is a potential greenhouse gas emitted from natural sources like wetlands, enteric fermentation in livestocks and human activities like landfills, coal mining, wastewater treatment plants and manure management to mention a few. Methanogens are obligate anaerobes present in diverse anoxic environment, forms an exclusive component of gut ruminants and have been found to have health implications in animals and recently reported in human hind gut and possible role in pathogenesis. The practical problem faced with the research in methanogens is the difficulties in isolation and detection of the organisms in the environmental samples and the in this regard antibodies may pave way for novel applications, Generation of antibodies from egg yolk have already been demonstrated to be more beneficial than other sources. Egg yolk antibodies can be generated by injecting whole cell suspensions of methanogen strains into the veins of white leg horn chickens followed by harvesting of egg yolk antibodies, their characterization, evaluating the IgY antibodies affinity to whole cell suspension and purified antigen, further optimization of antigen antibodies reaction and development of DOT-ELISA for detection of methanogens. It is already reported that the feeding of ruminants with anti-methanogens antibodies can reduce the load of methanogens in the gut of cattle that may lead to reduction in global methane emission

Introduction

A report by the Intergovernmental Panel on Climate Change (IPCC), convened by the United Nations, revealed that human activities over the past 50 years have influenced Global Climate through the emission of Green House Gases (GHG), which results in increased absorption in the atmosphere of infrared radiations emitted from the earth's surface. The accumulation of GHG results in increased global temperature (approximately 0.6 to 0.7°C), which in turn can increase annual precipitation in high rainfall regions and decrease precipitation in regions of low rainfall (Gerstengarbe & Werner, 2008). The most important GHG's are carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), which have increased in the last 150 years (Monteny *et al.*, 2006) and have different global warming potential. According to Ramaswany *et al.*, (2001) and Solomon *et al.*, (2007), the warming potential of CO_2, CH_4 and N_2O is 1, 23, and 298, respectively. Burning of fossil fuels is the main source of CO_2 emissions, while agriculture activities are the main contributors of global emissions of CH_4 and N_2O (Wheeler *et al.*, 2008). Thus, adoption of agricultural practices and technologies aimed specifically at reducing emissions from this sector will have a significant impact on total GHG emissions (Lascano and Cardenas, 2010). Hence there is a need to investigate how the CH_4 emissions from ruminants could be reduced. This paper focuses on enteric CH_4 and its mitigation using the Chicken IgY generated against ruminant methanogens.

Global Warming

Weather and climate have a profound impact on living organisms on the planet. Ecological systems have evolved over geological time scales to suit the prevailing climate. The past 10 to 20 years have brought disturbing evidence that human activities may cause significant changes in future global climate. "Global Warming" is now an issue known to hundreds of millions of people across the world. At this point it is useful to note the difference between weather and climate. Weather is the state of the atmosphere (temperature, humidity, precipitation, wind, cloud cover, etc.) in a particular location at a particular time; it fluctuates greatly and is notoriously difficult to predict. Climate is the time-averaged weather in a given geographical region. Climate is a temporal and spatial average and is consequentially much more predictable than weather. Thus, the average temperature during a given month in a particular area (climate) can be predicted with some confidence, however, the temperature at a given time and location (weather) is much more difficult to predict. Climate varies from month to month, season to season, and year to year. Statistically significant changes in climate occurring over a time scale of decades or longer constitute "climate change".

Global warming, an increase in Earth's near-surface temperature, is believed to result from the increase of "greenhouse gases." They could absorb outgoing infrared (heat) radiation and re-emit it back to Earth, warming the surface. Thus, these gases act like the glass of a greenhouse enclosure, trapping infrared radiation inside and warming the space. Global warming is an important environmental issue which is rapidly becoming a part of popular culture.

Green house effect and global climate change

The greenhouse effect is a natural process that plays a major part in shaping the earth's climate. It produces the relatively warm and hospitable environment near the earth's surface where

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humans and other life-forms have been able to develop and prosper. It is one of a large number of physical, chemical and biological processes that combine and interact to determine the earth's climate.

Climate, whether of the earth as a whole or of a single country or location, is often described as the synthesis of weather recorded over a long period of time. It is defined in terms of long-term averages and other statistics of weather conditions, including the frequencies of extreme events. Climate is far from static. Just as weather patterns change from day to day, the climate changes too, over a range of time frames from years, decades and centuries to millennia, and on the longer time-scales corresponding to the geological history of the earth. These naturally occurring changes, driven by factors both internal and external to the climate system, are intrinsic to climate itself. But not all changes in climate are due to natural processes. Humans have also exerted an influence. Through building cities and altering patterns of land use, people have changed climate at the local scale. Through a range of activities since the industrial era of the mid-19th century, such as accelerated use of fossil fuels and broadscale deforestation and land use changes, humans have also contributed to an enhancement of the natural greenhouse effect. This enhanced greenhouse effect results from an increase in the atmospheric concentrations of the so-called greenhouse gases, such as carbon dioxide and methane, and is widely believed to be responsible for the observed increase in global mean temperatures through the 20th century. The relationship between the enhanced greenhouse effect and global climate change is far from simple. Not only do increased concentrations of greenhouse gases affect the atmosphere, but also the oceans, soil and biosphere. These effects are still not completely understood. Also, complex feedback mechanisms within the climate system can act to amplify greenhouse-induced climate change, or even counteract it.

Green House Gases (GHG)

Although the earth's atmosphere consists mainly of oxygen and nitrogen, neither plays a significant role in enhancing the greenhouse effect because both are essentially transparent to terrestrial radiation. The greenhouse effect is primarily a function of the concentration of water vapour, carbon dioxide (CO_2), and other trace gases in the atmosphere that absorb the terrestrial radiation leaving the surface of the earth (IPCC 2001). Changes in the atmospheric concentrations of these greenhouse gases can alter the balance of energy transfers between the atmosphere, space, land, and the oceans. A gauge of these changes is called radiative forcing, which is a measure of the influence a factor has in altering the balance of incoming and outgoing energy in the earth-atmosphere system (IPCC 2001). Holding everything else constant, increases in greenhouse gas concentrations in the atmosphere will produce positive radiative forcing (i.e., a net increase in the absorption of energy by the earth).

Naturally occurring greenhouse gases include water vapour, CO_2, methane (CH4), nitrous oxide (N_2O), and ozone (O_3). Several classes of halogenated substances that contain fluorine, chlorine, or bromine are also greenhouse gases, but they are, for the most part, solely a product of industrial activities. Chlorofluorocarbons (CFCs) and hydrochlorofluorocarbons (HCFCs) are halocarbons that contain chlorine, while halocarbons that contain bromine are referred to as bromofluorocarbons (i.e., halons). Some other fluorine-containing halogenated substances—hydrofluorocarbons (HFCs), perfluorocarbons (PFCs), and sulfur hexafluoride (SF6)—do not deplete stratospheric ozone but are potent greenhouse gases. These latter substances are addressed by the UNFCCC and accounted for national greenhouse gas inventories.

There are also several gases that, although they have no commonly agreed upon direct radiative forcing effect, do influence the global radiation budget. These tropospheric gases include carbon monoxide (CO), nitrogen dioxide (NO2), sulfur dioxide (SO2), and tropospheric (ground level) O_3. Tropospheric ozone is formed by two precursor pollutants, volatile organic compounds (VOCs) and nitrogen oxides (NOx) in the presence of ultraviolet light (sunlight). Aerosols are extremely small particles or liquid droplets that are often composed of sulfur compounds, carbonaceous combustion products, crustal materials and other human induced pollutants. They can affect the absorptive characteristics of the atmosphere. Comparatively, however, the level of scientific understanding of aerosols is still very low (IPCC 2001). Among all the green house gases, the three most powerful long lived greenhouse gases in the atmosphere are carbon dioxide, methane, and nitrous oxide, their Global Atmospheric Concentration, Rate of Concentration Change, and Atmospheric Lifetime (years) are summarized in the table-1.

Table 1: The three most powerful long lived greenhouse gases in the atmosphere

Atmospheric Variable	CO_2	CH_4	N_2O
Pre-industrial atmospheric concentration	278 ppm	0.715 ppm	0.270 ppm
Atmospheric concentration	379 ppm	1.774 ppm	0.319 ppm
Rate of concentration change	1.4 ppm/yr	0.005 ppm/yra	0.26% yr
Atmospheric lifetime	50–200d	12e	114e

Source: IPCC (2007)

Methane and Its Significance in Global warming

Methane's contribution in global warming is 18% after CO_2 (66%) and followed by CFC (11%) and N_2O (5%) (Prasad and Rai, 2000). Thus, Methane is a potent green house gas second only to carbon dioxide as regards to its concentration by volume in the atmosphere. Methane has a global warming potential of 21 over a 100-year period. This means that on a kilogram for kilogram basis, methane is 21 times more potent than carbon dioxide during this time period. Its present concentration in atmosphere is 1.72 ppmv (which is double of its concentration in pre-industrial period) and increasing 0.015ppmv per year. On complete combustion, one kg of methane produces 55685 kJ of heat whereas, 2428 kJ and 6280 kJ of heat is produced from one kg of gunpowder and nitro-glycerine respectively (Misra, 1989). Thus, Methane is a very powerful source of energy. Natural sources of methane include wetlands, fossil sources, termites, oceans, freshwaters, and non-wetland soils. Methane is also produced by human-related (or anthropogenic) activities such as fossil fuel production, coal mining, rice cultivation, biomass burning, water treatment facilities, waste management operations and landfills, and domesticated livestock operations (ARM Facilities Newsletter, 2001).

Livestock are well-known to contribute to GHG emissions. In the widely - cited 2006 report (Livestock's Long Shadow) by the United Nations Food and Agriculture Organization (FAO), it is indicated that 18 % of annual worldwide GHG emissions, are attributable to cattle, buffalo, sheep, goats, camels, horses, pigs, and poultry. Agriculture and in particular enteric fermentation in ruminants (predominantly cattle and sheep) produces between 21 and 25% of the total anthropogenic emissions of CH_4 on a global scale.

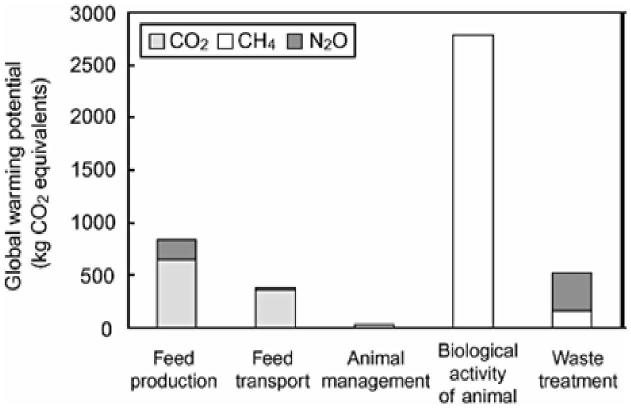
Table 2: Annual Global Methane Emission from Various Sources

S.No.	Source	Methane Emission (in thousand tons)
1	Live Stock	65-100
2	Rice	60-100
3	Natural Gas & Oil	32-68
4	Biomass Burning	28-51
5	Liquid Wastes	29-40
6	Coal	24-40
7	Landfills	20-28
8	Manure	8-18
9	Minor Industries	4

This source of methane is called enteric CH_4. The two major sources of agricultural CH_4 emissions are enteric fermentation in ruminants and livestock manure.

The production of greenhouse gases (GHG) from livestock is a major concern

(FAO 2006)) (Ogino *et al.*, 2007)



Enteric methane production in ruminants

Methane producing bacteria reside in the reticulo-rumen and large intestine of ruminant livestock. These bacteria, commonly referred to as methanogens, belong to the domain *Archaea* and the phylum *Euryarchaeota*. Unlike *Bacteria*, methanogens lack peptidoglycan in the cell wall, replaced by pseudomurein in *Methanobrevibacter* and *Methanobacterium*, heteropolysaccharide in *Methanosarcina*, and protein in *Methanomicrobium*. They use a range of substrates produced during the primary stages of fermentation to produce CH_4, thus creating generated energy required for their growth. All methanogen species can utilize hydrogen ions (H_2) to reduce CO_2 in the production of CH_4 as this reaction is thermodynamically favorable to the organisms. Availability of H_2 in the rumen is determined by the proportion of end products resulting from fermentation of the ingested feed. Processes that yield propionate and cell dry matter act as net proton-using reactions, whereas a reaction that yields acetate results in a net proton increase (Hegarty, 1999). Other substrates available to methanogens include formate, acetate, methanol, methylamines, dimethyl sulfide and some alcohols, however, only formate has been documented as an alternative methane precursor in the rumen (Jones, 1991).

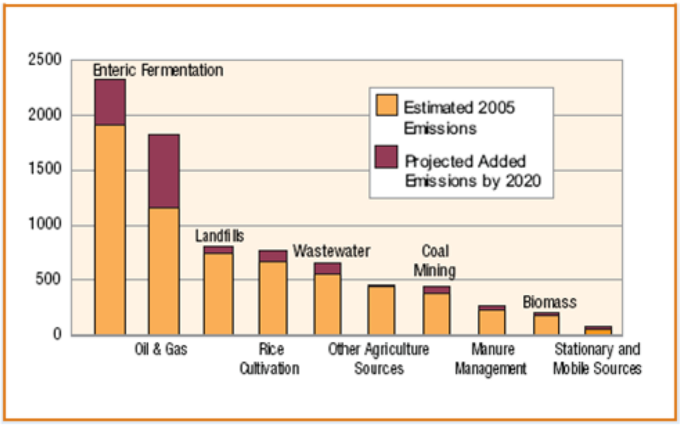
Table 3: Methane Emission Factors for Livestock and Manure

Animal Types	Enteric Fermentation, kg CH4/head/year	Manure Management, kg CH4/head/year
Bulls	75	1
Dairy Cows	118	36
Beef Cows	72	1
Dairy heifers	56	36
Beef Heifers	56	1
Heifers for slaughter	47	1
Steers	47	1
Calves	47	1
Sheep	8	0.19
Goats	8	0.12
Horses	13	1.4

(Environment Canada, 2002)

Global anthropogenic methane emissions are projected to increase by 23 percent to 7,904 MMTCO2 E by 2020

(Source: Bartos, 2009, US-EPA)



Factors Influencing Methane Production in Ruminants: a) Level of feed intake, b) Type of carbohydrate fed and c) Alteration of the ruminal microflora (Johnson & Johnson, 1995).

Feed consumed by cattle is fermented in the rumen by bacteria, protozoa, and fungi and as a result polysaccharides in the feed are converted into volatile fatty acids (VFA) and microbial protein accompanied by the release of gaseous by-products (carbon dioxide and hydrogen) (Kamra, 2005). In adult cattle molecular hydrogen is produced every day, which does not accumulate as gases in the rumen given the presence of methanogenic archaea and other hydrogen utilizing microbes in the rumen. The symbiosis between bacteria that ferment polysaccharides and produce hydrogen and the methanogens which utilize hydrogen to reduce CO_2 and produce CH_4 results in an enhanced digestion of feed and microbial biomass production. As a result of this process, ruminants' loose between 2–12% of the gross dietary energy in the form of CH_4, depending on the quality and quantity of diet offered and consumed (Johnson & Johnson, 1995). Approximately 87% of the enteric CH_4 is produced in the rumen and the remaining 13% is released in the large intestine through fermentation (Lockyer & Jarvis 1995; Lassey *et al.*, 1997). Thus it is essential to look for alternatives to reduce CH4 from Live-stock emissions and by doing so contribute to less GHG.

Methane Mitigation

A human intervention to reduce the sources or enhance the sinks of greenhouse gases is referred to as mitigation; methane mitigation is the human interference to reduce the methane concentration in the atmosphere. The methane is removed naturally from the atmosphere in three ways. These methods, commonly referred to as sinks, are oxidation by chemical reaction with tropospheric hydroxyl ion, oxidation within the stratosphere, and microbial uptake by soils. In spite of their important role in removing excess methane from the atmosphere, the sinks cannot keep up with global methane production. Since, enteric methane (CH4) is the most important contributor of GHG emissions in ruminant production, it is essential to look for alternatives to reduce CH4 from Live-stock emissions and by doing so contribute to less GHG. Methane mitigation is effective in one of two ways: either a direct effect on the methanogens, or an indirect effect caused by the impact of the strategy on substrate availability for methanogenesis, usually through an effect on the other microbes of the rumen.

Many mitigating strategies proposed have indeed multiple modes of action including chemical suppression and biotechnological interventions have been investigated to attenuate methane production and improve feed efficiency. However, there is growing concern over the use of chemical inhibitors in animals used for human consumptions, and possibility in developing chemical resistant methanogens, researches are now focusing on developing biological strategies to solve the problem. Wright *et al*., (2004) conducted an in vivo assessment of two formulations of methanogen vaccines in sheep to reduce methane emissions. They reported that of two vaccines tested, the formulation with fewer antigenic targets resulted in a significant (7.7%) reduction of methane emissions compared with a control group, the significant decrease in methane emissions observed were due to specific activity of anti-methanogen secretory antibodies delivered to the rumen via saliva. Complete genome sequencing of methanogens can also immensely help in developing vaccine and small molecular inhibitors and presently the option of selection and breeding of low methane emitting animals are also a viable process under review (Leahy *et al*., (2013).

Chicken Egg Yolk (IgY) antibodies in Methane Mitigation

On the basis of the influence of methanogen vaccines in the reduction of rumen methane emission and a number of researchers also have shown that IgY antibodies can be used for passive immunization or treatment of animals suffering from various bacterial and viral diseases, researchers are focussing on a novel adaptation of passive immunization by oral administration of hen egg yolk antibody (IgY) to control methane emission. It may achieve the similar or better results as like the specific antibodies induced by methanogen vaccines. Additionally, passive delivery of antibodies will prevent some of the adverse reactions observed in response to immunizations, including temporary reduction to the animal's live weight and local reactions at the site of immunization. Furthermore, the yolk of eggs from laying hens immunized with the target antigen is shown to be an inexpensive and convenient source for polyclonal antibodies.

Characteristics of Egg Yolk Antibodies (EYA)

The presence of immunoglobulins in eggs is an example of passive immunity because these antibodies are derived from the dam and protect the offspring from various infectious diseases after hatch (Hatta *et al*., 1997). The acquisition of passive immunity in birds was first noted in 1893 when Klemperer showed the transfer of immunity to tetanus toxin from hen to chick (Rose and Orlans, 1981). Three immunoglobulin classes (IgA, IgM, and IgY) are present in chickens (Karlsson *et al*., 2004). Leslie and Clem (1969) proposed that chicken IgG be designated as IgY, as it is different from mammalian IgG and forms the main immunoglobulin in chickens (Leslie and Clem 1969; Leslie *et al*., 1971). IgY is transported from the hen to the embryo via the egg yolk, which, as a result contains high concentrations of this antibody (Hamal *et al*., 2006). Other immunoglobulin classes are present in negligible amounts in the egg yolk (Carlander *et al*., 1999) and IgY is not present in the egg white (Rose *et al*., 1981). A laying hen can produce approximately 300 eggs annually, and each egg yolk volume is approximately 15 ml (Wilkie, 2006). The amounts of IgY in yolk are 20-25 mg/ml (Rose and Orlans, 1981), which would supply over 100 g of antibody per hen per year. There are several reports in the literature indicating that IgY levels in the egg yolk are not always consistent and may vary within and between bird populations. (Yegani and Korver, 2010)

Avian (IgY) anti-methanogen antibodies for reducing ruminal methane production

Cook *et al*., (2008) have reported that antibodies from hen's egg can decrease the methane production *in vitro*. They assessed *in vitro* dry matter disappearance (IVDMD) and production of methane, volatile fatty acids (VFA) and ammonia from an early lactation diet or from freeze-dried alfalfa in the presence of anti-methanogen antibody treatments in two *in vitro* ruminal incubations (experiments 1 and 2). In experiment 1, hens were immunised with crude cell preparations of *Methanobrevibacter smithii*, *Methanobrevibacter ruminantium* or *Methanosphaera stadtmanae* and complete Freund's adjuvant (CFA). Semipurified egg antibodies (IgY) prepared from the immunised hens' eggs (α-SMI^{CFA}, α-RUM^{CFA}, or α-STAD^{CFA}, respectively) were dispensed into 24 replicate vials (400 µL per vial) containing 500 mg of an early lactation total mixed ration (18% crude protein; 33% neutral detergent fibre; DM basis). Vials containing an equal volume of semi-purified antibodies from eggs of non-immunised hens were included as a control. In experiment 2, hens were immunised with one of the three antigenic preparations combined with Montanide ISA 70 adjuvant. Triplicate vials per time point included 0.6 g of freeze-dried egg powder (α-SMI^{Mon}, α-RUM^{Mon}, α-STAD^{Mon}; 19.0 ± 2.6 mg IgY/g) or a mixture of all three (Combo^{Mon}) and 500 mg of freeze-dried alfalfa. Total gas, methane production and pH were measured at intervals over 24 h. After 24 h, samples were analysed for VFA, ammonia and IVDMD. In experiment 1, cumulative CH_4 production was similar ($P > 0.05$) among treatments at each sampling time. At 24 h, average CH_4 production across treatments was 27.03 ± 0.205 mg/g DM. In experiment 2, α-SMI^{Mon}, α-STAD^{Mon} and Combo^{Mon} reduced methane production at 12 h ($P \le 0.05$) compared with the control, but by 24 h, CH_4 levels in all treatments were similar ($P > 0.05$) to the control. At 24 h, total VFA concentrations were lower ($P < 0.05$) in α-RUM^{Mon} and α-SMI^{Mon} than in the control. The transient nature of the inhibition of methane production by the antibodies may have arisen from instability of the antibodies in ruminal fluid, or to the presence of non-culturable methanogens unaffected by the antibody activity that was administered.

More recently Pradip Maiti, (2010, US7820171B2) investigated the role of avian antibodies directed against the methanogens in ruminant methane production *in vitro*. In this study avain antibody responses induced by immunization of chickens with methanogenic antigens such as *M. smithi*, *M. ruminantium* and *M. stadtmaniae* formulated with appropriate adjuvant [CFA/IFA and mineral oil (MONTANIDE ISA 70)]. Then the hyper immunized eggs were collected and utilised for antibody purification, finally the effects of the treatment with PBS, non-immunized egg powder (antibody control) and specific anti-methanogen antibodies individually and three antibodies (anti-*M. stadtamaniae*, anti-*M. smithii* and anti-*M. stadtmaniae*) in combination on methane production in *in vitro* ruminal fermentation were assessed at 12-hours post-treatment. When compared with the antibody control and PBS control, there was a significant difference in reduction of methane production at 6, 12 and 24-hours post-treatment using anti-methanogen antibodies, in particular, the results demonstrated that the maximum reduction in methane production was achieved with the treatment of ruminant fluid with the three anti-methanogne antibody combination in *in vitro* fermentation.

Conclusion

The above recent findings indicate that, specific anti-methanogen avian antibodies can be generated following immunization of chickens with the optimal dose of methanogen formulated with an appropriate adjuvant and the anti-methanogen antibodies have the capacity to prevent methanogenesis in the rumen. Furthermore, the most dramatic effect on reduction of methane production has been achieved in the treatment with combination of the three anti- methanogen antibodies rather single antibodies. Therefore, it can be concluded on the basis of the above findings, an intervention strategy can be developed using the avian anti-methanogen antibody combination to reduce ruminal methane emissions. Although beneficial effect of anti-methanogen antibodies found that, their role in the reduction of methane emission by *in vito* ruminant fermentation, further research is needed to adapt this new strategy for methane mitigation. Because the methanogens are obligate anaerobes and are fastidious to culture in laboratory conditions. It is well recognized that our inventory of culturable species may not represent the diversity of the ruminant's resident methanogens population. Molecular analyses estimating phylogenetic diversity of ruminant methanogens have revealed multiple clusters of related methanogens. Therefore, a combination of antibodies targeting individual strains may be the most appropriate, broad spectrum approach to reduce methane production.

In summary, the reduction of methane emission in ruminants is possible through various strategies. Today, the feeding management approach is the most developed. Other strategies such as feed additives and anti-methanogen antibodies are promising but the diversity of the rumen bacterial and methanogenic communities may be a limiting factor for their successful application. In any case, before practical solutions are projected for field application more research *in vivo* and time are required. The sustainability of methane mitigation strategies is an important issue.

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