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SUPPLEMENTS TO REDUCE METHANE PRODUCTION IN RUMINANT ANIMALS

abdulaziz Almaghrabi
abdulaziz.almaghrabi@siu.edu

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by

Abdulaziz M. Almaghrabi

B.S., King Abdulaziz University at Saudi Arabia, 2012

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Submitted in Partial Fulfillment of the Requirements for
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RESEARCH PAPER APPROVAL

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in the field of Animal Sciences

Approved by:

Dr. Amer AbuGhazaleh, Chair

Dr. Jayakrishnannair Puthenpurayil Sasidharannair

Dr. Amir Sadeghpour

Graduate School
Southern Illinois University Carbondale
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AN ABSTRACT OF THE RESEARCH PAPER

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TITLE: SUPPLEMENTS TO REDUCE METHANE PRODUCTION IN RUMINANT ANIMALS

MAJOR PROFESSOR: Dr. Amer AbuGhazaleh

Changes in the climate continue to be one of the world's most pressing issues. The accumulation methane (CH_4) in the atmosphere is a major driver of these changes. Ruminant animals have been identified as substantial contributors to CH_4 emissions. This assertion is supported by the fact that ruminant animals have a unique digestive tract that allows them to digest complex polysaccharides by the action of microbes in their rumen. During the microbial digestion (fermentation) of dietary polysaccharides, CH_4 is produced as a byproduct of the process. Therefore, this paper aims to analyze this issue, highlight how ruminants contribute to this phenomenon and suggest measures that can be implemented to mitigate CH_4 production by these animals. Research studies in recent years have tested if different supplements may reduce CH_4 emissions from ruminant animals by suppressing some of the methane-producing microbes in the rumen or by altering the hydrogen (H_2) utilization in the rumen. Results in this paper showed that some of the tested supplements (e.g. Ionophores, tannins, essential oils (EO), nitrate, bioflavonoids, and enzymes) or their combinations can decrease CH_4 production in the rumen without significantly affecting animal's performance or rumen fermentation. However, the results were not always consistent depending on the supplement dosage and treatment diet composition. Although some of the tested suppleemnts showed promising effects, more research is still needed to assess the long-term effects for some of these supplements as rumen microbial adaptation

may weaken the reported effects.

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HEADING 1

INTRODUCTION

Climate change continues to be one of the most significant problems facing the contemporary world. The accumulation of greenhouse gases such as carbon dioxide and CH_4 in the atmosphere, which block the Sun's light and contribute to these changes in climate and temperature patterns, is a significant cause of these alterations. In recent years, CH_4 emissions have been a significant source of concern. Methane accounted for approximately 11% of all greenhouse gases emitted in the United States in 2020, primarily due to human activities (the United States Environment Protection Agency, 2022). Leaks in natural gas systems and animal husbandry are two of the most prevalent contributors to CH_4 emissions. Natural sources such as natural wetlands and chemical reactions in the atmosphere also produce CH_4. Studies have shown that CH_4 warming potential is 25 times greater than carbon dioxide's (the United States Environment Protection Agency, 2022). However, CH_4 has a much shorter lifetime than carbon dioxide, as it can only remain in the atmosphere for up to 12 years. In contrast, carbon dioxide can be retained for more than a century (the United States Environment Protection Agency, 2022).

Methane is primarily a natural gas, but its danger is linked to its ability to trap atmospheric heat causing global warming. So, instead of escaping into space, solar energy reaches the earth's surface is reflected as longer-wavelength infrared radiations and this event contributes to climate change by causing the atmosphere to warm (Hertzberg et al., 2017). Currently, the weather and temperature patterns around the globe are changing. These alterations have led to a rise in global sea levels and desertification and an increase in the frequency of natural disasters such as hurricanes, floods, and earthquakes. These issues pose a substantial risk to human lives and the be environment. Given the substantial effects of these greenhouse gases, their emission levels must regulated.

It has been determined that ruminant animals are significant contributors to CH_4 emissions. This claim is based on the fact that ruminant animals have a unique digestive system that permits them to digest complex dietary carbohydrates (e.g. cellulose). Such abilities are not observed in many other animal species. The rumen contains a variety of microorganisms, including bacteria, fungi, and protozoa, which degrade dietary carbohydrates (starch and fiber) through a process called hydrolysis (fermentation) to produce volatile fatty acids (VFA) such as acetate, propionate, and butyrate (Shokryzadan et al., 2016). However, during the same processes, varying amounts of formic acid, hydrogen (H_2), and carbon dioxide are produced as byproducts of the fermentation process. According to Danielsson et al. (2017), most methanogenic archaea in the rumen use H_2 to reduce carbon dioxide and produce CH_4. This process maintains a low partial pressure of H_2, thereby directing fermentation toward the production of less reduced byproducts, such as acetate.

Ruminant animals emit a substantial quantity of CH_4 into the atmosphere. According to Shokryzadan et al. (2016), ruminant enteric fermentation accounts for between 15 and 20% of global CH_4 emissions and approximately 4% of total greenhouse gas production. In ruminant animals, the production of these gases results in a loss of between 11 and 13% of the digestible energy (Shokryzadan et al., 2016). Therefore, methods to effectively manage CH_4 production in ruminant animals are required.

Different methods have been used to manage CH_4 emissions; however, dietary manipulations still represent the primary key strategy. For example, Garcia et al. (2020) suggested that the use of ionophores (antibiotics) in diets, could enhance feed efficiency. This additive modulates the activities of specific microorganisms in the rumen, and different research indicated that ionophores can reduce CH_4 emissions and improve energy utilization in beef and dairy cattle

(Garcia et al., 2020). However, Durmic et al. (2014) noted that even though some of the antibiotics (ionophores) could reduce CH_4 emissions, this is not the case for all antibiotics and the effect may not be persistent over time due to rumen adaptation (Embaby et al., 2018). Additionally, with the emergence of antibiotic resistance in human pathogens, the use of safer anti-methanogenic components such has essential oils (EO) and extracts from plants is becoming important.

Methane gas has major effects on the environment, so its production by ruminant animals must be regulated. A key method of achieving the above is through manipulating animal diets to reduce the amount of CH_4 produced. One of the significant suggested ways to manipulate rumen fermentation is using plant extracts and EO. In the following part, a brief review of some research articles classified by supplement types will be discussed.

HEADING 2

IONOPHORES

Monensin was tested as an ionophore-feed supplement for its ability to reduce enteric CH_4 output and renewable methanogenesis (Takahashi & Iwasa, 2021). Using Holstein-Friesian steers, a respiratory trial with a head cage was conducted to elucidate the suppressive action of monensin. Ad libitum high-concentrate meals (80% concentrate and 20% hay) with or without monensin, galactooligosaccharides (GOS), or L-cysteine were provided to the steers. When compared to steers fed control diets, calves given diets containing monensin or GOS considerably reduced their CH_4 emissions (Takahashi & Iwasa, 2021). A study by Crossland (2017) investigated the effects of feed additive rotation on CH_4, VFA, and microbial population response. For 13 weeks, cannulated steers (n = 12) were fed a moderate forage basal diet and along with the regular feed, steers were randomly assigned to one of six treatments (control, 20 mg bambermycin, 200 mg Monensin, the basal diet+ weekly rotation of Monensin and bambermycin (BM7), the basal diet + rotation of bambermycin and monensin treatments every 14 days (B14M), and the basal diet+ rotation of Monensin and bambermycin treatment every 21 days (BM21). Rumen fluid was collected weekly for analysis, and the results were standardized to the individual's organic matter intake (OMI; kg/d). The result of the experiment demonstrated that the capacity for CH_4 production was not substantially different across treatments. On the other hand, treatments affected the CH_4–to–propionate ratio, with the control value being the greatest and the Monensin, B21M, and B14M values being the lowest (0.42 vs. 0.36, 0.36, and 0.33, respectively). Additionally, the CH_4: propionate ratio was lowest in weeks 2 and 3 but remained constant in weeks 4 to 12. The week also affected total VFA, with a peak in week three and a decline in week four (4.02 vs. 2.86 mM/kg OMI). The acetate-to-propionate (A:P) ratio demonstrated a significant, treatment-week interaction, with steers given bambermycin or rotationally having a higher A:P ratio than steers fed Monensin throughout the feeding period. The author concluded that although ionophores have distinct mechanisms of action, there is no evidence that rotating bambermycin and Monensin alters the VFA profile or reduces CH_4 potential more than continuous feeding dilutes the ionophore's efficacy when administered continuously.

Another study by Witzig et al. (2018) looked at whether changes in the abundances of cellulolytics and methanogens occurred along with the CH_4-decreasing effects of Monensin and other hydrolyzable tannins during the *in vitro* fermentation of grass silage. Two rumen simulation approach tests were performed in which grass silage was either evaluated in combination with Monensin (0, 2 or 4 mg d^1) or with various tannin extracts from chestnut, valonea, sumac, and grape seed (0 or 1.5 g d^1) (Witzig et al., 2018). Using paraformaldehyde-ethanol-fixed cells, total prokaryotes were measured, and the relative abundances of ruminal cellulolytic and methanogenic species were evaluated using real-time quantitative PCR. Results showed that compared to control, Monensin administration had no effect on the absolute numbers of prokaryotic cells but supplementation with grape and chestnut seed tannins reduced overall prokaryotic counts. Methane production decreased by 21% with Monensin and by 65 and 24% with the chestnut and grape seed tannins, respectively. The relative abundances of all methanogens, however, were similar across tannin treatments. Chestnut and valonea tannins inhibited R. *albus* while the relative abundance of F. *succinogenes* reduced with the addition of Monensin. Furthermore, supplementation with Monensin or chestnut tannins was observed to decrease the relative abundances of *Methanobrevibacter* sp., particularly M. *ruminantium*, and *Methanosphaera stadtmanae*. The study findings showed that Monensin and tannins have different effects on ruminal cellulolytics and that monensin and chestnut tannins are linked with lower levels of

M. *ruminantium* and M. *stadtmanae.*

Another study by Vyas et al. (2018) investigated the impact of monenison in reducing CH_4 production in ruminant animals. The purpose of this research was to assess the combined effects of feeding backgrounding and finishing beef calves with monensin (MON) and the CH_4 inhibitor 3-nitrooxypropanol (NOP) on enteric CH_4 emissions, growth rate, and feed conversion efficiency. In a 238-day feeding trial, 240 crossbred steers were placed on a backgrounding diet for the first 105 days (backgrounding phase), followed by transition diets for 28 days, and finally a finishing diet for the final 105 days (finishing phase). Fuor treatments were administered: control , monensin supplemented (MON) at 33 mg/kg DM, 3-nitrooxypropanol (NOP) at the backgrounding phase 200 mg/kg DM or in the finishing phase at 125 mg/kg DM, and MONOP (33 mg/kg DM MON supplemented with either 200 mg/kg DM or 125 mg/kg DM NOP). Randomized full blocks (heavy and light weights) were used in the experiment with a 2 (NOP) 2 (MON) factorial arrangement of treatments utilizing 24 pens (8 cattle/pen; 6 pens/treatment) at the main feedlot and 8 pens (6 cattle/pen; 2 pens/treatment) at the controlled environment building (CEB) feedlot. During both stages, five animals per treatment were transferred to chambers for CH_4 measurements. Overall, there were little interactions between MON and NOP, showing that their effects were distinct. When cattle were given the backgrounding diet, pen DMI dropped by 7%, but gain-to-feed ratio increased by 5% with NOP supplementation. Likewise, MON enhanced the G:F ratio by 4%, but had no effect on DMI. During the concluding phase, DMI decreased by 5% with both MON (5%) and NOP (5%), while ADG decreased by 3% with MON. With NOP, the gain-to-feed ratio for finishing cattle increased by 3% but MON had no impact. 3-Nitrooxypropanol lowered CH_4 yield (g/kg DMI) by 42 and 37%, respectively, in backgrounding and finishing diets, but MON had no effect on CH_4 production. These findings reveal the effectiveness of NOP in lowering CH_4

emissions and, therefore, enhancing feed conversion efficiency in cattle given high-forage and high-grain diets. In addition, the effects of NOP were independent of the inclusion of MON in the diet.

HEADING 3

NITRATE, SAPONIN, AND SULFATE

A study by Sun et al. (2016) investigated whether Cysteamine hydrochloride (CHS) and nitrogen oxide (NO_3-) were able to inhibit CH_4 production and improve the growth performance of cattle. The study was done under both *in vitro* and *in vivo* conditions. Rumen fluid was collected from two rumen fistulated steers approximately two hours after giving 1 kg of concentrate and *ad libitum* Chinese ryegrass (*Leymus chinensis*). The effects of NO_3 and CHS on live weight gain (LWG), DMI, feed conversion ratio (FCR; feed:gain), daily CH_4 production (DMP), and CH_4 yield (MY) of calves were investigated using a completely randomized design in comparison to a control group fed the identical roughage/concentrate diet. The results of the *in vitro* experiment showed CHS did not affect CH_4, total VFA, and acetate production nor the acetate:propionate ratio, although it did lower the pH and increased H_2 buildup compared to the control. In the *in vivo* experiment, CHS supplemented cattle had no differences in LWG or FCR compared to cattle fed the control diet while reducing daily CH_4 production. While neither DMP nor MY (CH_4/kg DMI) were lowered by CSH, CH_4 generation rate was significantly decreased for up to 6 h post-feeding in comparison to control animals. Nitrate on the other hand decreased MY by 31.1% (15.7 g CH_4/kg DMI) compared to the control diet (22.8 g CH_4/kg DMI). Authors concluded that NO_3 can provide better CH_4 mitigation than CSH.

Another study by Patra and Yu (2014) assessed the effects of nitrate (NO_3-; sodium nitrate), sulfate (potassium sulfate), and Quillaja saponin (derived from the bark of Quillaja Saponaria Molina plants) on CH_4 production, feed fermentation, and the abundance of microorganisms in the rumen of Jersey cows. The Quillaja saponin product had a sapogenin concentration of 24%. Quillaja saponin (0.6 g/L), NO_3- (5 mM), and sulfate (5 mM) were employed separately or in two-and three-way combinations, yielding eight treatments: control (no methanogenic inhibitor), saponin, NO_3-, sulfate, sulfate plus saponin, and NO_3- plus sulfate and saponin. The results of this experiment indicated that the combinations of NO_3-, saponin, and sulfate suppressed CH_4 production additively, with the lowest reduction (nearly 46%) observed when all three inhibitors were combined. None of the treatments had a detrimental effect on feed digestion, rumen fermentation, total bacteria, Ruminococcus albus, or Archaea abundances. However, Quillaja saponin increased the abundance of *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* when used alone or in combination with NO_3- and sulfate but decreased the abundance of protozoa. The researchers concluded that the combination of the above additives is a possible mitigation measure against CH_4 production in ruminants, and they can be used in animal feeds while ensuring animals suffer minimal impacts.

Another study by Petersen et al. (2015) investigated whether increasing the intake of dietary NO_3- would also decrease the potential for CH_4 emissions in ruminant animals. Because NO_3- can act as an alternative H_2 sink, supplementing ruminant diets with it has been proposed as a CH_4 mitigation strategy. A balanced Latin square design assigned four rumen- and intestinal-cannulated Holstein cows to the experiment. The cows were fed one of four diets over four weeks, all of which were similar except for the gradual replacement of urea with NO_3 - in the form of Bolifor (Yara), a pelleted but readily soluble compound containing >70% NO_3. The final diets had NO_3- contents of 0 (NO3-0), 5 (NO3-1), 14 (NO3-2), and 21 g NO_3 - kg^{-1} DM. (NO3-3). Dietary NO_3 – decreased N_2O emissions from dairy cows, but only at higher NO_3 intake levels of 14 and 21 g kg DM. Supplementing diets with NO_3^- reduced CH_4 emissions relative to the control diet, but the reduction levels were very small (less than 2%). The researchers concluded that dietary NO_3^- caused decreased N_2O emissions from dairy cows; as a result, which might partially offset the CH_4 mitigation potential of dietary NO_3^-.

HEADING 4

ESSENTIAL OILS, BIOFLAVONOIDS, AND TANNINS

A study by Garcia et al. (2020) investigated the effectiveness of EO of *L. turbinata* and *T. minuta* in reducing CH_4 production during the *in vitro* fermentation of substrates that are representative of different livestock production systems. Two independent trials were conducted using the same methodology to evaluate each EO. Four typical simulated diets of livestock production systems—breeding, rearing, fattening, and dairy—were evaluated. The EO doses were 0, 0.3, 3, 30, and 300 mL/L. Monensin treatment at 1.87 mg pure monensin/L was also included for each substrate. There were no differences in the variables at the low dosages (0.3 and 3 ml/L). Except for a substantial decrease (8.7%) in the OMD for the fattening substrate and a slight reduction (3.6%) in the NDFD for the normal substrate, the intermediate dose (30 mL/L) reduced gas and CH_4 production without influencing substrate digestibility. The maximum dose of EO (300 ml/L) resulted in a significant drop in gas and CH_4 generation, although this was not connected with a significant decrease in substrate digestibility. The researchers concluded that EO could reduce CH_4 production, but their effectiveness depends on the dose level and the interactions between the fermented substrates.

Rossi et al. (2022) investigated the efficacy of a blend of bioflavonoids and tannins on CH_4 emissions and the production efficiency of dairy cows. For this study, two different trails were performed which aimed to evaluate the efficacy of a coated blend of EO, mainly from coriander seed (*Coriandrum sativum*), cloves (*Syzygium aromaticum*), and geranium (*Pelargonium cucullatum*), tannins (CT) from chestnuts (*Castanea sativa*) and bioflavonoids (BF) from olives (*Olea europea*). The relative concentration of the active ingredients in the product was set as follows EO: CT: BF = 1:2.5:0.1. The researcher tested the blend under *in vitro* conditions to examine the potential to reduce CH_4 production when used under standardized conditions. The blend was also tested *in vivo*, and in this case, lactating dairy cows were used to evaluate the effects it had on milk production and the apparent total tract digestibility of the diet. The mixture considerably decreased *in vitro* total gas and CH_4 emissions at 16, 20, and 24 hours. Additionally, after 24 h, the concentration of acetate decreased while that of propionic acid increased. In the *in vivo* study, the treatment group demonstrated a significant increase in milk yield, DMI, FCR, and the total tract digestibility for cellulose and starch. The researchers concluded that blending EO, bioflavonoids, and tannins helped reduce CH_4 production and improved the production efficiency of the lactating dairy cows.

A study by Samal et al. (2016) investigated the effects of dietary supplementation of combinations of different plants containing tannins, saponins, and EO on the growth performance, nutrient utilization, and methanogenesis in growing buffaloes. Twenty buffalos were divided into four treatment groups and fed either a basal diet consisting of wheat straw and concentrated mixture (control) or the control diet plus Mix-1 (ajwain oil and lemongrass oil in 1:1 ratio at 0.05% of DMI), Mix-2 (garlic and soapnut in 2:1 ratio at 2% of DMI) and Mix-3 (garlic, soapnut, harad, and ajwain in 2:1:1:1 ratio at 1% of DMI). The experiment was eight months long, however, after 130 days of feeding, a metabolism trial was performed using an open-circuit indirect respiration calorimeter to measure CH_4 emission from animals. The result of the experiments showed that buffaloes' feed intake and growth pattern were unaffected by dietary supplements. The feed conversion ratio (FCR) was higher in Mix-1, Mix-2, and Mix-3 than in control by 9.5, 7, and 10.2%, respectively. Apart from CP digestibility, the digestibility of other nutrients was comparable across all groups. Methane emissions (L/kg DMI and L/kg digestible DMI) were reduced by 13.3 and 17.8% in the Mix-1, 10.9 and 13.5% in the Mix-2, and 5.1 and 9.8% in the

Mix-3 groups, respectively, when compared to the control. The study concluded that feeding phytogenic feed additives might help reduce CH_4 production without affecting feed utilization.

Benchaar (2016) investigated the impact of cinnamon oil (CIN), cinnamaldehyde (CDH), or monensin (MON) supplements in dairy cow diets on the enteric production of CH_4, ruminal fermentation, protozoa population, digestion, nitrogen balance, and milk performance. A total of eight multiparous lactating Holstein cows fitted with ruminal cannulas were used in the experiment. Cows were either fed a control diet or a diet supplemented with CIN (50 mg/kg DM intake), CDH (50 mg/kg DM intake), or MON (24 mg/kg DM intake). Dry matter intake, nutrient digestibility, N retention, CH_4 emissions, and milk performance were measured over six consecutive days. Rumen degradability of the diet was assessed using in sacco incubations at 0, 2, 4, 8,16, 24, 48, 72 and 96 hours. The results of this experiment revealed that CIN, CDH, or MON diet supplementation had no impacts on the DMI, N retention, sacco ruminal degradation, and nutrient digestibility. Further, enteric emission of CH_4 was not affected by the addition of CIN, CDH, or MON to the diet. The author concluded that CIN, CDH, and MON could not be promoted as effective strategies to mitigate the enteric CH_4 production in dairy cows.

Another study by Poornachandra et al. (2019) investigated performing a comparison of the effects of individual or combined supplementation of condensed tannins (*Tamarindus indica*) and saponins-containing (*Sapindus mukorossi*) Phyto-sources on CH_4 production in cattle. The researcher selected 24 animals which were then divided into four groups with an equal number and then fed with finger millet straw as well as a concentrated based diet as outlined: T (tamarind seed husk supplemented group), S (soapnut supplemented group), and TS (tannins/saponins; tamarind seed husk and soapnut combined supplementation in 60:40). The basal diet consisted of 51 g/kg of tamarind seed husk and soapnut, either individually (T, S) or combined (TS). The *in* *vivo* study indicated that there was a significant reduction in the enteric CH_4 emission by almost 20% when tamarind seeds husk or the combination of supplementation of tamarind seed husk and soapnut were used. The authors concluded that tamarind seed husk alone or the combination with soapnut at the above level could help establish a CH_4 emission low diet, but there is a need to confirm the efficacy of the diet in reducing the emission levels by performing long trials on ruminants.

HEADING 5

FIBROUS SUBSTRATE

Li et al. (2014) examined the long-term effects of the Eremophila glabra, a native Australian shrub, on rumen fermentation characteristics, in particular, CH_4 productions and the methanogen population. The control diet mix contained 780 g/kg DM oaten chaff, 280 g/kg DM lupin grain, and 20 g/kg DM mineral. Three levels of *E. glabra* were tested: EG15 (150 g/kg DM), EG25 (250 g/kg DM), and EG40 (400 g/kg DM), and the treatments were prepared by substituting a portion of the control diet mix with *E. glabra*. Each treatment had three replicates, and all treatments were randomly assigned to the fermentation vessels. The results of the experiment showed that CH_4 production reduced, and the overall CH_4 reduction was 32 and 45% for EG15 and EG25, respectively, compared to the control. The researcher also noted that on day seven, fermentation was virtually entirely suppressed in the EG40 treatment (thus, the result of CH_4 production was not provided). Their results also showed that the total bacterial numbers were not altered, but the population of methanogen population decreased by about 42.1% (EG40) compared to the control diet. Additionally, *Fibrobacter succinogenes* population also reduced considerably at all levels of *E. glabra*. However, *Albus* population was reduced only with the EG40 diet. The study concluded that substituting a portion of a fibrous substrate with E. glabra can reduce CH_4 production.

A study by Kumar et al. (2022) investigated the impact of dietary phytogenic composite feed additives that contain a mixture of dried leaves of *P. deltoides* and *E. citriodora* on growth performance and nutrient utilization in buffalo (*Bubalus bubalis*) calves. The study was done in two stages. In the first phase, the researcher did an *in vitro* study which involved a rumen fermentation process where they aimed to identify the most suitable blend concentration of poplar

(*P. deltoides*) and eucalyptus (*E. citriodora*) leaf-meal that can reduce CH_4 production without affecting the degradability of the feed. The second phase involved an *in vivo* study to assess the dietary inclusion of the above blend on the production performance of the buffalo calves. Eighteen female Murrah buffalo calves (*Bubalus bubalis*, avg. BW 131 ±7.5 kg, 10–14 months old) were randomly divided into three groups of six animals each and fed control, EPLM-1, and EPLM-2 (eucalyptus (*Eucalyptus citriodora*) and poplar (*Populus deltoides*) leaf-meal. Each buffalo calf in the treatment groups (EPLM-1 and EPLM-2) was fed EPLM at either 1.3 or 3.9% of feed dry matter intake by combining it with a small portion of concentrate mixture, which was offered daily at 10:30 a.m. The experimental feeding lasted 100 days, with 10 days of dietary adaptation and 90 days of actual data recording and measurement. The study results indicated that the average daily weigh gain and the final body weight of all the experimental buffalo were similar across experimental groups. The enteric CH_4 production was reduced in EPLM supplemented buffaloes; however, the digestibility of all the nutrients remained comparable among the animals. The researcher concluded that blends of eucalyptus and poplar leaf-meal (50 g/h/d) containing 3.19 g, 2.30 g, and 0.71 g of total phenolics, tannin phenolics, and condensed tannins, respectively, can be utilized as a phytogenic feed additive to improve mitigate enteric CH_4 production without affecting performance and nutrient utilization.

Shokryzadan et al. (2016) investigated the mangosteen peel's (MP) effects on *in vitro* ruminant fermentation, gas production, CH_4 production, and microbial population. The study composed of three treatments including the control (C, 50% concentrate + 50% alfalfa without MP); medium MP (MMP, 50% concentrate + 25% alfalfa + 25% MP); and high MP (HMP, 50% concentrate + 50% MP). Their results showed that CH_4 production for MMP (18.3 mL CH_4/g DM) and HPM (18.2 mL CH_4/g DM) were lower than that of the control group (19.9 mL CH_4/g

DM). Moreover, the study showed that MP reduced CH_4 production per unit of total gas production (0.08 ± 0.01 and 0.08 ± 0.00 for the MMP and HMP groups, respectively) in comparison with 0.09 ± 0.01 for control group. Finally, authors reported no effects of MP inclusion on VFA production and *Butyrivibrio fibrisolvens* population. The researchers concluded that MP has the potential of reducing ruminal CH_4 production without negatively affecting rumen fermentation.

HEADING 6

ENZYMES

A study by Hernández (2017) investigated the impact of supplementing calves' diets with exogenous enzymes (xylanase; XYL) and yeast (*Saccharomyces cerevisiae* [SC]) on the long-term control of CH_4 production. The researcher tested the supplementation of three different levels of XYL (0, 3, and 6 mg/g of DM), SC (0, 2, and 4 mg/g of DM), and a mixture of XYL and SC (0, 2 mL XYL 2 mg SC, 6 mL XYL 4 mg SC/g of DM). The results of the experiment showed that asymptotic gas production (GP) was consistently reduced by each additive, with the lowest value at the high dose of XYL SC mixture when equated to the control and the low dose of XYL SC mixture. Relative to the control treatment, including the above additives reduced CH_4 production with the high dose had the most significant reduction in GP and CH_4 production compared to the control, XYL, and SC additives at different doses. The authors concluded that XYL or SC additives could improve rumen fermentation and lower greenhouse gas emissions. The researchers also found that when XYL and SC are combined, they become more effective at reducing gas and CH_4 emissions for relatively clean environmental conditions in calf farming.

HEADING 7

SULPHUR SUPPLEMENTS

Uniyal et al. (2020) investigated the impact of Sulphur feeding on the CH_4 production, nutrient utilization, and growth performance of goats. The researchers selected fifteen goats and divided them into three equal groups using a random sampling technique. The group named T1 was utilized as the control group and Sulphur supplement was added at 0.08 and 0.16% of diet DM to the animals in groups T2 and T3. The experiment was carried out for sixty days. The results of this study showed that the intake and the digestibility of DM, OM, cruid protein (CP), ether extract (EE), neutral detergent fiber (NDF), and acid detergent fiber (ADF) were not impacted by the Sulphur supplementation. Nitrogen metabolism in goats was also not affected by the Sulphur supplements. However, a 12.3% reduction in CH_4 production was noted due to dietary supplementation of Sulphur in T3 as compared to the control group. Nevertheless, the energy partitioning was not affected at all levels. The researchers concluded that the dietary supplementation of Sulphur might help reduce CH_4 emission in ruminants, but higher doses using a larger pool of animals need to be tested to reach a significant level.

HEADING 8

CONCLUSION

Methane emission has reached worrisome levels, and ruminants have been identified as a major contributor to the dramatic increase in its atmospheric levels. It is noted that these animals have complex digestive systems that enable them to digest feeds with a high cellulose content, but in doing so; they produce CH_4 gas, which poses a severe environmental threat due to its potent properties. In this research paper, we looked at the use of different feed additives or feed sources for their potential effects on rumen CH_4 production as well as rumen fermentation and animals' performance. The products examined in this research paper generally helped in reducing the amount of CH_4 produced but with variable effects on rumen fermentation and animals' performance.

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VITA

Graduate School

Southern Illinois University

Abdulaziz M. Almaghrabi

aalmaghrabi69@gmail.com

King Abdulaziz University at Saudi Arabia, Jeddah
Bachelor of Science, Arid Land Agriculture, 2012

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Major Professor: Dr. Amer AbuGhazaleh