Asian-Aust. J. Anim. Sci.
Vol. 22, No. 7 : 1043 - 1047
July 2009

www.ajas.info

In vivo Methane Production from Formic and Acetic Acids in the Gastrointestinal Tract of White Roman Geese

Yieng-How Chen[1], Shu-Yin Wang[2] and Jenn-Chung Hsu*
Department of Animal Science, National Chung Hsing University, Taichung, 402 Taiwan

ABSTRACT : Three experiments were conducted to determine the conversion rate of formic and acetic acids into methane in the gastrointestinal tracts of geese. In experiment I, two sets of two 4-month-old male White Roman geese were allocated to one of two treatment groups. Each set of geese was inoculated either with formic acid or with phosphate buffer solution (PBS). After the acid or the PBS was inoculated into the esophagi of the geese, two birds from each treatment were placed in a respiratory chamber as a measurement unit for 4 h in order to determine methane production rate. In experiment II and III, 6- and 7-wk-old male White Roman goslings were used, respectively. Birds were allocated to receive either formic acid or PBS solution injected into the ceca in experiment II. Acetic acid or PBS solution injected into the cecum were used for experiment III. After either the acids or the PBS solution were injected into the cecum, two birds from each treatment were placed in a respiratory chamber as a measurement unit for 3 h; each treatment was repeated 3 times. The results indicated that formic acid inoculated into the oesophagi of geese was quickly converted into methane. Compared with the PBS-injected group, methane production increased by 5.02 times in the formic acid injected group (4.32 vs. 0.86 mg/kg BW/d; $p<0.05$). Acetic acid injected into the ceca did not increase methane production; conversely, it tended to decrease methane production. The present study suggests that formic acid may be converted to methane in the ceca, and that acetic acid may not be a precursor of methane in the ceca of geese. (**Key Words :** Acetic Acid, Formic Acid, Gastrointestinal Tract, Geese, Methane)

INTRODUCTION

Excess release of greenhouse gas into the atmosphere is detrimental to the environment and may contribute to global climate change. The main contributors of greenhouse gases emitted from livestock and poultry are methane (CH_4) and nitrous oxide (N_2O) that are produced during enteric and manure fermentation. Geese are herbivorous poultry; their ceca possess cellulase activity (Chen et al., 1992) and can digest cellulose (Yang and Lin, 1975). The cecum is the major fermentation site in birds (Annison et al., 1968; McBee, 1969; Clemens et al., 1975). The main fermentation products in the ceca of birds are short-chain-fatty acids that include acetic, propionic and butyric acids (Chen et al., 1992) as well as gases such as methane (Marounek et al., 1999; Tsukahara and Ushida, 2000; Chen et al., 2003) and carbon dioxide (Gasaway, 1976). Previous studies indicated that the methane production of caecectomized geese was only 8-10% of that of sham-operated geese (Chen et al., 2003); this result demonstrated that the ceca of geese can produce methane. In ruminants, some researches have been conducted to investigate methane production by *in vitro* (Lee et al., 2003; Kumar et al., 2007) and in the rumen (Bhatta et al., 2007). It has been indicated that formic acid can be converted into methane in the rumen (Hungate, 1966; Czerkawski, 1986). Therefore, it is reasonable to speculate that the same activity may be found in geese as they are herbivorous birds. However, there is little information available concerning the chemical process of methane production in the ceca of goslings. The purpose of this study, therefore, was to measure the methane production rate as well as the conversion rate of formic and acetic acids to methane in the gastrointestinal tract of goslings.

* Corresponding Author: Jenn-Chung Hsu. Tel: +886-4-2285 0315, Fax: +886-4-22860265, E-mail: jchsu@dragon.nchu.edu.tw
[1] Department of Animal Science and Biotechnology, Tunghai University, 181 Taichung Harbor Road, Taichung, 406 Taiwan.
[2] Department of Animal Science, Chinese Culture University, 55 Hwa-Kang Road, Taipei, 111 Taiwan.
Received June 4, 2008; Accepted November 27, 2008

MATERIALS AND METHODS

Experimental protocol

Experiment I. Animal management and experimental *treatments* : Four, 4-month-old male White Roman geese with similar body weights were selected from our experimental geese group reared in National Chung Hsing University and divided into two groups: one group had formic acid solution inoculated into the esophagi of birds (experimental group) while the other group was inoculated with PBS solution (control group). The birds were kept on a high wire floor pen in a windowless house and fed a commercial pellet diet (crude protein 16.1%; ME 2900 kcal/kg). Feed and water were supplied *ad libitum*.

After the geese were fasted for 4 h, the experimental group was inoculated with 7.2 ml of 4.08% formic acid solution (0.3 ml of 98% formic acid (Sigma) added to 6.9 ml of 0.75 M phosphate buffer, pH adjusted to 6.80 with 10 N NaOH) via oral injection using a 10 ml syringe. The control group geese were inoculated with 7.2 ml of 0.75 M PBS (pH 6.80) in the same manner. Thereafter, the birds were re-fed for 1 h. Subsequently, two birds from each group were placed into a respiratory chamber (Chen et al., 2002) for 4 h. The gas released from enteric fermentation in the birds was collected and analyzed for methane. Triplicate gas samples were collected from a sample outlet using a gas-tight syringe at 0, 1, 2, 3, and 4 h.

Experiment II. Animal management and experimental treatments : Experiment II used twelve, 6-wk-old White Roman goslings. The birds were fed a commercial pellet diet (crude protein 18.9%; ME 2,970 kcal/kg) and allocated to either a control group (ceca injected with PBS) or an experimental group (ceca injected with formic acid). In both control and experimental groups, there were three cages (90 cm×60 cm×60 cm) containing 2 goslings each. Feed and water were supplied *ad libutum*.

Formic acid solution was injected into the ceca of the birds. Methane production from enteric fermentation was measured by placing goslings (three runs per treatment group, 2 birds per run) in a respiration chamber. Goslings were fasted for 5 h and re-fed for 5 h before the cecal injection operation. The ceca could be found via a 4-5 cm abdominal midline incision (Chen et al., 2002). Each side of the cecum was injected with 1.2 ml of formic acid solution (4.08% formic acid in 0.75 M phosphorus buffer, pH adjusted to 6.80 with 10 N NaOH). In the control group, 1.2 ml of PBS was injected. After the cecal injection, the skin and muscle layers were sutured. Then, two birds from each group were placed into a respiratory chamber for 3 h. This was repeated 3 times for each group at 6 wks of age. The gas released from the enteric fermentation in the birds was collected and analyzed for methane. Triplicate gas samples were collected from a sample outlet using a gas-tight syringe at 0 and 3 h.

Experiment III. Animal management experimental treatments : Twelve, 7-wk-old White Roman goslings were fed a commercial pellet diet (crude protein 18.9%; ME 2,970 kcal/kg) and allocated to either control (ceca injected with PBS) or experimental groups (ceca injected with acetic acid). In both control and experimental groups, three cages (90 cm×60 cm×60 cm) contained 2 goslings each. Feed and water were supplied *ad libutum*.

In experiment III, acetic acid solution was injected into the ceca. Methane production as a result of enteric fermentation was measured by placing goslings in a respiration chamber at a rate of three runs per treatment group, 2 birds per run. The management and surgical treatment of goslings were identical to experiment II. Each side of the cecum was injected with 1.2 ml acetic acid solution (4.08% acetic acid in 0.75 M phosphate buffer, pH adjusted to 6.80 with 10 N NaOH). In the control group, 1.2 ml of PBS was injected. After the cecal injection, the skin and muscle layers were sutured. Then, two birds from each group were placed in a respiratory chamber for 3 h. This was repeated 3 times for each group at 7 wks of age. The gas released as a result of enteric fermentation was collected as indicated for experiment II.

Gas analysis

Methane was analyzed using gas chromatography (Shimadzu, model 14 B) with a flame ionization detector. The column was a Porapak Q (2 m inner diameter 1/8 inch, Supelco; PA, USA); the oven temperature was 70°C, the injection temperature was 130°C and the detector temperature was 130°C. Nitrogen was used as the carrier gas; the flow rate was 10 ml/min. The reference methane gas (95.5%, Chinese petroleum Co.) was diluted to 10, 50, 100, 500 and 1,000 ppm with nitrogen (98.5%) for the construction of a standard curve. The methane concentration of each sample was calculated from the standard curve (Wang et al., 2003; Wang and Huang, 2005).

Statistical analysis

Data were analyzed by an analysis of variance utilizing the general linear model (GLM) procedure. All statistical analysis was carried out by SAS software (SAS, 1996). The least square means were used to compare and estimate the difference ($p<0.05$) between the two treatments in each experiment.

RESULTS

Experiment I. The conversion of formic acid into methane in the gastrointestinal tract

The effect on methane production of formic acid inoculation into the esophagi of 4-month-old White Roman geese is presented in Table 1. Methane production increased with time in both control and treated birds. The formic acid-inoculated group showed higher cumulative methane production than the control group at each sampling from 1

Table 1. Effect of formic acid inoculation into esophagus on cumulative methane production in 4-month-old White Roman geese

Time (h)	Control (PBS inoculated)	Formic acid inoculated
	------------- Methane production (mg/bird) --------	
0	1.79±0.02	1.73±0.01
1	2.22±0.10 [a]	2.88±0.07 [b]
2	2.80±0.07 [a]	4.05±0.06 [b]
3	3.34±0.09 [a]	4.87±0.17 [b]
4	3.69±0.04 [a]	5.36±0.10 [b]

[a, b] Means within the same row with different superscripts differ significantly ($p<0.05$).

to 4 h ($p<0.05$). The maximum methane production rate was between 1 and 2 h after the birds were placed into the respiratory chamber (Table 2). The formic acid-treated birds had a greater ($p<0.05$) methane production rate during the first 3 h following treatment.

Experiment II. The conversion of formic acid into methane in the ceca

In experiment II, the daily methane production per bird in the formic acid-treated group was 4.93 times higher than in the control group (11.88 vs. 2.41 mg/bird/day, $p<0.05$). Moreover, the methane production per kg of body weight in the formic acid-treated birds was 5.02 times (4.32 vs. 0.86 mg/kg BW/day, $p<0.05$) higher than in the control group (Table 3). In comparison with the control group, there was a significantly ($p<0.05$) higher methane production rate (mg/bird/h or mg/kg BW/h) in the formic acid-treated birds.

Experiment III. The conversion of acetic acid into methane in the ceca

Both methane production and the methane production rate in goslings injected in the ceca with acetic acid were numerically less than in goslings injected with PBS (Table 4). However, no significant difference was observed between the two treatments.

DISCUSION

Experiment I. The conversion of formic acid into methane in the gastrointestinal tract

Table 2. Effect of formic acid inoculation into esophagus on methane production rate in 4-month-old White Roman geese

Period (h)	Control (PBS inoculated)	Formic acid inoculated
	------ Methane production rate (mg/bird/h) -------	
0-1	0.43±0.11[a]	1.15±0.08[b]
1-2	0.58±0.16[a]	1.17±0.03[b]
2-3	0.54±0.07[a]	0.82±0.23[b]
3-4	0.35±0.09	0.49±0.23

[a, b] Means within the same row with different superscripts differ significantly ($p<0.05$).

Table 3. Effect of formic acid injection into ceca on methane production in 6-week-old White Roman goslings

	Control (PBS inoculated)	Formic acid injected
Methane production		
mg/bird/d	2.41±0.44[a]	11.88±2.82[b]
mg/kg BW/d	0.86±0.16[a]	4.32±1.02 [b]
Methane production rate		
mg/bird/h	0.10±0.02[a]	0.50±0.12 [b]
mg/kg BW/h	0.04±0.01[a]	0.18±0.04 [b]

[a, b] Means within the same row with different superscripts differ significantly ($p<0.05$).

Geese that had been administered formic acid into the esophagus showed greater methane production than those administered PBS (Expt. I). Thus, formic acid can be converted into methane in the gastrointestinal tract. Formic acid is a precursor of methane in the rumen (Hungate et al., 1970). Vercoe and Blaxter (1965), and Blaxter and Czerkawski (1966) demonstrated that methane production increased when formic acid was inoculated into sheep. On the other hand, methane production is increased when formic acid is added to the rumen fluid of cattle (Beijer, 1952). Methane production in the formic acid-inoculated group of geese was already greater than in the control group after 1 h. This implies that formic acid inoculated into the esophagus could be quickly converted into methane. The maximum methane production rate occurred between 1 and 2 h after inoculation; subsequently the methane production rate decreased. The decreased methane production over time suggested that the substrate (formic acid) had decreased and perhaps the formic acid inoculated into the esophagi of geese could be quickly converted into methane.

Experiment II. The conversion of formic acid into methane in the ceca

Goslings with formic acid injected into their ceca produced a greater methane volume than the control group. This result was similar to experiment I; after the formic acid inoculation into the esophagus, methane production was enhanced. The data suggest that the cecum is the site for methane production in geese. In ruminants, the site of formic acid-enhanced methane production is the rumen

Table 4. Effect of acetic acid injection into ceca on methane production in 7-week-old White Roman goslings

	Control (PBS inoculated)	Acetic acid injected
Methane production		
mg/bird/d	6.05±2.19	3.96±1.69
mg/kg BW/d	2.02±0.58	1.37±0.56
Methane production rate		
mg/bird/h	0.25±0.09	0.16±0.07
mg/kg BW/h	0.08±0.02	0.06±0.02

(Beijer, 1952; Vercoe and Blaxter, 1965; Blaxter and Czerkawski, 1966). The mechanism of methane production from formic acid in the ceca of geese may be similar to the mechanism in the rumen.

The quantity of formic acid inoculated in experiment I was higher than that injected in experiment II. However, the methane production on a per bird per day basis for the formic acid inoculated group was only 1.45 times that of the control group (32.16 vs. 22.14, calculated from Table 1) in experiment I. In experiment II however, the methane production per day per bird for the formic acid-injected group was 4.93 times that of the control group. This phenomenon may result from: i) Not all of the formic acid inoculated into the esophagus entered the cecum. Gasaway et al. (1976) showed that only 86% of intestinal fluid enters the ceca; ii) A portion of formic acid was absorbed in the small intestine before it entered the ceca; iii) A portion of formic acid was excreted from the cloaca. The possibility of excretion is suggested because the passage rate of the digesta in the alimentary canal in goslings ranges from only 130 min to 198 min (Chen et al., 1991) and the ceca of the bird becomes almost completely empty only once every 24 hours (Mattocks, 1971; Duke et al., 1984).

Experiment III. The conversion of acetic acid into methane in the ceca

Acetic acid is the major volatile fatty acid in the ceca of goslings (Chen et al., 1992). However, methane production did not increase in goslings injected with acetic acid in the cecum. In fact, methane production tended to be suppressed. This suggests that acetic acid in the ceca is not a precursor of methane. Decreasing methane production has been previously shown when acetic acid was added to peat (Williams and Crawford, 1984), inoculated into sheep (Armstrong and Blaxter, 1957; Blaxter and Czerkawski, 1966) and added to the rumen fluid of cattle (Beijer, 1952). The reason for the adverse effect of acetic acid on methane production in this study is unclear. Perhaps acetic acid is not decomposed as quickly as formic acid by the methanogenic bacteria in goslings, resulting in a decrease in pH. Van Kessel and Russell (1996) indicated that ruminal methanogenesis was inhibited by the toxicity of fermentation acids at low pH. Russell (1992) noted that volatile fatty acids such as acetic acid, were lipophylic compounds in their un-dissociated form which easily traversed cell membranes. However, the internal pH is more alkaline than the external pH; the acetic acid will cross the membrane, dissociate and accumulate intracellularly, as well as increase volatile fatty acid anions (Van Kessel and Russell, 1996). This may account for toxicity of acetic acid to methanogenic bacterium and inhibition of their growth (Nieman, 1954). Whether there is a similar inhibition of the methanogenesis mechanism by acetic acid in both ruminants and birds, the present study suggests that further research is needed to resolve this problem.

CONCLUSIONS

Formic acid inoculated into the esophagi of geese can be rapidly converted into methane. Compared to the PBS injected into the cecum of birds, methane production was increased in formic acid injected birds. Acetic acid injected into the cecum of birds did not increase methane production, but tended to decrease it. Based on these results, it is concluded that formic acid can be converted into methane in the ceca of goslings and is a precursor of methane.

ACKNOWLEGMENTS

The authors would like to thank Miss Chi Yin Chiou, Yang-Ting Hsiao and Mr. Wen Wan Kuo at the department of Animal Science, Chinese Culture University for their assistance in animal management, sample collection and gas determination.

REFERENCES

Annison, E. F., K. J. Kill and R. Kenworthy. 1968. Volatile fatty acids in the digestive tract of the fowl. Br. J. Nutr. 22:207-216.

Armstrong, D. G. and K. L. Blaxter. 1957. The increasement of steam-volatile fatty acids in fasting sheep. Br. J. Nutr. 11:247-274.

Beijer, W. H. 1952. Methane fermentation in the rumen of cattle. Nature 170:576-577.

Bhatta, R., O. Enishi and M. Kurihara. 2007. Measurement of methane production from ruminants. Asian-Aust. J. Anim. Sci. 20:1305-1318.

Blaxter, K. L. and J. Czerkawski. 1966. Modifications of the methane production of the sheep by supplementation of its diet. J. Sci. Food. Agric. 17:417-421.

Chen, Y. H., J. C. Hsu and L. L. Lu. 1991. The effect of high and low dietary fiber level on digesta passage through alimentary canal of goslings. Tunghai J. 32:765-774 (in Chinese).

Chen, Y. H., J. C. Hsu and B. Yu. 1992. Effects of dietary fiber levels on growth performance, intestinal fermentation and cellulase activity of goslings. J. Chin. Soc. Anim. Sci. 21(2): 15-28 (in Chinese).

Chen, Y. H., F. M. Pan and J. C. Hsu. 2002. The caecectomy of geese. Taiwan Vet. J. 28:74-79 (in Chinese).

Chen, Y. H., S. Y. Wang and J. C. Hsu. 2003. Effects of caecectomy on body weight gain, intestinal characteristics and enteric gas production in goslings. Asian-Aust. J. Anim. Sci. 16:1030-1034.

Clemens, E. T., C. E. Stevenes and M. Southworth. 1975. Site of organic acid production and pattern of digesta movement in gastrointestinal tract of geese. J. Nutr. 105:1341-1350.

Czerkawski, J. W. 1986. An introduction to rumen studies. Pergamon Press, New York, USA. pp. 185-219.

Duke, G. E., E. Eccleston, S. Kirywood, C. F. Louis and H. P.

Bedbury. 1984. Cellulose digestion by domestic turkeys fed low and high fiber diets. J. Nutr. 114:95-102.

Gasaway, W. C. 1976. Cellulose digestion and metabolism by captive rock ptarmigan. Comp. Biochem. Physiol. 54A:179-182.

Gasaway, W. C., R. C. White and D. F. Holleman. 1976. Digestion of dry matter and absorption of water in the intestine and cecum of the ptarmigan. Conder 78:77-84.

Hungate, R. E. 1966. The Rumen and Its Microbes. Academic Press, New York, USA. pp. 133-273.

Hungate, R. E., W. Smith, T. Bauchop, I. Yu and J. C. Rabinowitz. 1970. Formate as an intermediate in the bovine rumen fermentation. J. Bacteriol. 102:389-397.

Kumar, R., D. N. Kamra, N. Agarwal and L. C. Chaudhary. 2007. *In vitro* methanogenesis and fermentation of feeds containing oil seed cakes with rumen liquor of buffalo. Asian-Aust. J. Anim. Sci. 20:1196-1200.

Lee, H. J., S. C. Lee, J. D. Kim, Y. G. Oh, B. K. Kim, C. W. Kim and K. J. Kim. 2003. Methane production potential of feed ingredients as measured by *in vitro* gas test. Asian-Aust. J. Anim. Sci. 16:1143-1150.

Marounek, M., O. Suchorska and O. Savka. 1999. Effect of substrate and feed antibiotics on *in vitro* production of valatile fatty acids and methane in caecal contents of chickens. Anim. Feed Sci. Technol. 80:223-230.

Mattocks, J. G. 1971. Goose feeding and cellulose digestion. Wildfowl 22:107-113.

McBee, R. H. 1969. Cecal fermentation in the willow ptarmigan. Conder 71:54-58.

Nieman, C. 1954. Influence of tract amounts of fatty acids on the growth of the microorganisms. Microbiological Reviews 18: 147-163.

Russell, J. B. 1992. Another explanation for the toxicity of fermentation acids at low pH: Anion accumulation versus uncoupling. J. Appl. Bacteriol. 73:363-370.

SAS. 1996. SAS user's guide: statistics. SAS Inst., Inc., Cary, NC. USA.

Tsukahara, T. and K. Ushida. 2000. Effects of animal or plant protein diets on cecal fermentation in guinea pigs (*Cavia porcellus*), rats (*rattus norvegicus*) and chicken (*Gallus gallus domesticus*). Comp. Biochem. Physiol. 127A:139-146.

Van Kessell, J. A. S. and J. B. Russell. 1996. The effect of pH on ruminal methanogenesis. FEMS Microbiol. Ecol. 20:205-210.

Vercoe, J. E. and K. L. Baxter. 1965. The metabolism of formic acid in sheep. Br. J. Nutr. 19:523-530.

Wang, S. Y. and D. J. Huang. 2005. Assessment of greenhouse gas emissions from poultry enteric fermentation. Asian-Aust. J. Anim. Sci. 18(6):873-878.

Wang, S. Y., S. W. Shieh, S. H. Wang and Y. H. Chen. 2003. Assessment of enteric fermentation emission factors of greenhouse gases in goose utilizing a respiratory chamber. J. Chin. Soc. Anim. Sci. 32:43-50 (in Chinese).

Williams, R. T. and R. L. Crawford. 1984. Methane production in Minnesota peatlands. Appl. Environ. Microbiol. 47:1266-1271.

Yang, C. P. and C. H. Lin. 1975. The utilizing of dietary fiber feed in geese. II. The function of cellulose digestion in ceca. J. Chin. Soc. Anim. Sci. 4:41-46 (in Chinese).