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# Methane Generation from the Intestine of Muscovy Ducks, Mule Ducks and White Roman Geese

**Yieng-How Chen[1], Shin-Mei Lee[2], Jenn-Chung Hsu[3], Yu-Cheng Chang[4], Shu-Yin Wang[5*]**

[1] *Department of Animal Science and Biotechnology, Tunghai University, No. 1727, Sec. 4, Taiwan Boulevard, Xitun District, Taichung 40704, Taiwan*
[2] *Department of Food Science, National Quemoy University, 1 University Road, Jinning Township, Kinmen, Taiwan*
[3] *Department of Animal Science, National Chung Hsing University, 250 Kao-Kung Road, Taichung 402, Taiwan*
[4] *Department of Environmental Engineering, National Cheng Kung University, 1 University Road, Tainan 70101, Taiwan*
[5] *Graduate Institute of Biotechnology, Chinese Culture University, 55 Hwa-Kang Road, Taipei 111, Taiwan*

## ABSTRACT

Livestock are one of the largest single sources of methane emissions, which is a greenhouse gas. In this study, alfalfa meal was tested as a feed additive to lower the methane generation in the three species of waterfowl. Four experiments were conducted to determine methane generation in the intestines of Muscovy ducks, mule ducks and White Roman geese of the same age and with the same diet. In experiment I, the *in vitro* methane generation of the cecal contents was the largest compared to other intestinal compartments in each species ($p < 0.05$). Moreover, the methane generation of the cecal content or colon and rectum in goslings was higher than in mule and Muscovy ducklings ($p < 0.05$) at six weeks of age. In experiment II, the cumulative methane generation in the cecal content of mule ducks at 8 weeks of age was the largest among all species ($p < 0.05$). In experiment III, 4-month-old Muscovy ducks had the highest average methane generation per bird per day among the three species ($p < 0.05$). In experiment IV, the methane generation of the cecal content in birds fed a grower diet supplemented with a 30% alfalfa meal diet was lower than that of birds fed grower diet for all three species. Based on the above results, it is suggested that age, the method of measurement (*in vitro* vs. *in vivo*) and composition of diet significantly affect the level of methane generation in the intestine for Muscovy ducks, mule ducks and White Roman geese. The amounts of methane generation of the cecal contents in the three species were 36.1, 13.9, and 34.2 µg/g/4 h, respectively. By adding 30% alfalfa meal, the cumulative methane generation of the cecal contents for the three species were reduced by 63.3–95.5%. The addition of alfalfa meal to feed thus has good potential with regard to decreasing greenhouse gases emissions from waterfowl.

***Keywords:*** Methane; Mule ducks; Muscovy ducks; White Roman geese.

## INTRODUCTION

The majority of the world's domestic waterfowl is in southeast Asia, with the domesticated duck population in these regions, accounting for 75% of the global total (Hetzel, 1985). The duck and goose industries are well developed in Taiwan, and are the country's second and third largest poultry industries, respectively. Ducks and geese are hindgut fermenters, and the major fermentation site is the caecum in birds (Annison *et al.*, 1968; McBee, 1968; Clemens *et al.*, 1975), and the main fermentation products in ceca are short chain fatty acids, e.g., acetic acid, propionic acid and butyric acid (Chen *et al.*, 1992) as well as gases, e.g., methane ($CH_4$) (Chen *et al.*, 2003a) and carbonic oxide (Gasaway, 1976). Carbon dioxide ($CO_2$) and methane are both "greenhouse gas," and their accumulation in the atmosphere has been cited as a factor in global warming (Van Kessell and Russell, 1996; Wang *et al.*, 2003). Methane is a significant greenhouse gas with a relatively short atmospheric lifetime of about 12 years, and is released to the atmosphere by biological processes occurring in anaerobic environments; moreover, the methane is second in importance only to $CO_2$ with regard to its environmental effects, and its relative global warming ability is 23 times that of $CO_2$ over a time horizon of 100 years (Jasim *et al.*, 2012).

Livestock are one of the largest single sources of methane emissions, with 80–115 million tonnes per year, equivalent to 15–20% of total anthropogenic methane (IPCC, 2001). The enteric fermentation emission factors of methane for geese are $1.50 \times 10^{-3}$ kg/head/life cycle (Wang *et al.*, 2003).

[*] Corresponding author.
Tel.: +886-2-2861-0511 ext. 31801; Fax: +886-2-2861-3100
*E-mail address:* sywang@faculty.pccu.edu.tw

However, the methanogenesis of waterfowl has rarely been reported, and there have been no studies published that compare methane generation in ducks and geese. Ducks are omnivorous birds while geese are herbivorous ones, and both can utilize forage. Alfalfa is a kind of good forage being full of nutrients which can benefit poultry, and thus alfalfa is often used as feed in the commercial waterfowl industry. Hu *et al.* (2005) show that tea saponin could decrease the resulting methane generation in ruminal fermentation *in vitro*. Holtshausen *et al.* (2009) note that, *in vitro*, increasing the level of two saponin sources, *Yucca schidigera* and *Quillaja saponaria*, can decrease methane generation. Alfalfa contains 0.8% saponin (Heywang and Bird, 1954), and thus it is worth examining whether adding alfalfa meal to the birds'basal diet could change the level of methane generation. The purpose of this study was to investigate the methane generation in different compartments of the intestine at different growth stages among Muscovy ducks, mule ducks and White Roman geese. In addition, the alfalfa was tested as a feed additive to lower the methane generation in the three kinds of waterfowl.

## MATERIALS AND METHODS

### *Animal and Management*

Ninety one-day-old apparently healthy mule and Muscovy ducklings and White Roman goslings, thirty of each species, were purchased from a commercial farm in Yung-Lin County, in southern Taiwan, and were used as experimental animals, which were kept in electric brooders from 0 to 2 weeks of age. All the birds were kept with each species separated in a high wire floor pen in a closed house (area 1.5 m × 2 m). At 0–3 weeks of age the birds were fed starter pelletted diets (crude protein: 19.3%; metabolizable energy; ME, 2900 kcal/kg) and after 3 weeks with grower and finisher diets (crude protein 16.1%, ME 2900 kcal/kg). Feed and water were supplied *ad libitum* throughout entire the experimental period. The following were four experiments carried out in this study, and summary in Table 1:

**Experiment I**: The main purpose of this experiment was to study the methane generation in various parts of intestine among three kinds of poultry. Six birds were selected randomly from each species at 6 weeks of age, and sacrificed by jugular vein bleeding after 4 hours fasting and then 6 hours resumed feeding. The contents of the duodenum, jejunum, ileum, cecum, colon and rectum of each bird were sampled and each was mixed in separated plastic bag, and then 0.3 g of intestinal content from each compartment was put into a 15 mL vial, containing 3.7 mL mixed nutritive buffer solution (Salvador *et al.*, 1993). The vials were then filled with 100% $CO_2$, and sealed with a butyl rubber stopper and aluminum clip. The samples were then incubated under anaerobic conditions at 38°C for 4 hours. At the end of the incubation, 0.2 mL 10% chloride mercury ($HgCl_2$) was added to vials to terminate bacteria activity and the gas was sampled from the vial with a gas-tight syringe to measure the methane concentration.

**Experiment II:** Caecum has been recognized as the major site of microbial digestion in poultry. Therefore, the isolated cecal content is used to verify that methane generation from enteric fermentation is contributed mainly by cecal fermentation. Six birds were selected randomly from each species at 8 weeks of age, and then sacrificed and the cecal contents sampled and treated as in experiment I. The samples were incubated under anaerobic conditions at 38°C for 0, 0.5, 1, 2, 3 and 4 hours. At the end of each incubation time, 0.2 mL 10% chloride mercury ($HgCl_2$) was added to terminate bacteria activity, and the gas was sampled from the vial with a gas-tight syringe to measure the methane concentration.

**Experiment III**: The aim of experiment **III** was to study the comparison of *in vivo* methane generation among Mule ducklings, Muscovy ducklings and White Roman goslings and to confirm the consistency with *in vitro* outcome in experiment II. Two 4-month-old mule ducks, Muscovy ducks and White Roman geese of each species were put into a closed respiratory chamber (90 cm × 60 cm× 60 cm) after fasting for 4 hours by rotation run, and then resumed feeding for 1 hour by rotation run. The air conditions and temperature of the respiratory chamber were set as in Chen *et al.* (2003a). The gas released from enteric fermentation in the birds was accumulated in the chamber, and was collected via a sample outlet using a 35 mL gas-tight syringe at 0 and 4 hour of the experiment. Samples were injected into a 15 mL serum bottle previously filled with $N_2$ using a replacing method. Five samples were collected at each sampling time and stored at room temperature for methane analysis.

**Experiment IV**: The aim of experiment **IV** was to study the effects of alfalfa included diet on the *in vitro* methane generation of the cecal contents among Mule ducklings, Muscovy ducklings and White Roman goslings. Thirty 6-week-old goslings, mule duckling and Muscovy duckling were divided into two groups, one fed with grower diets (CP 16.1%, ME 2900 kcal/kg) (Table 2) and the other with grower diets supplemented with 30% alfalfa (alfalfa: 17.3% CP and 24% CF) (3 species × 2 diets). In each treatment the goslings were allocated to one pen (1.5 m × 1.8 m) of five goslings each. Feed and water were supplied *ad libutum*. At 8 weeks of age, the birds were fasted for 8 hours and then resumed feeding for 8 hours before they were sacrificed by jugular vein bleeding. Cecal contents were collected from three birds in each treatment. Cecal content from the

**Table 1.** Experimental design for sampling and analysis.

| Experiment | Age for Sampling | Type of study | Methane generation source |
|---|---|---|---|
| I. | 6-week-old | *In vitro* | Contents from various compartments of intestine |
| II. | 8-week-old | *In vitro* | Ceca content |
| III. | 4-month-old | *In vivo* | Total gastrointestinal tract |
| IV. | 6-week-old | *In vitro* | Ceca content |

**Table 2.** Composition of experimental diets.

| Ingredients | Period | |
|---|---|---|
| | Starter (0–3 weeks of age) | Grower & finisher (after 4 weeks of age) |
| | ----------------%---------------- | |
| Yellow corn | 63.63 | 67.02 |
| Wheat bran | 3.60 | 8.00 |
| Soybean meal,44% | 25.2 | 17.00 |
| Corn gluten | 1.00 | 2.00 |
| Fish meal, 60% | 3.00 | 1.00 |
| Molasses | - | 1.50 |
| Dicalcium phosphate | 1.20 | 1.25 |
| Calcium carbonate, pulverized | 0.84 | 0.80 |
| Salt | 0.30 | 0.30 |
| DL-Methionine | 0.15 | 0.07 |
| Choline chloride, 50% | 0.08 | 0.06 |
| Premix* | 1.00 | 1.00 |
| Total | 100 | 100 |
| Calculated composition | | |
| Crude protein,% | 19.30 | 16.10 |
| ME, kcal/kg | 2900 | 2900 |
| Calcium, % | 0.88 | 0.82 |
| Available phosphorus, % | 0.44 | 0.41 |

* Supplied per kilogram of diet: vitamin A, 18,000IU; vitamin $D_3$, 2,250IU; vitamin E, 22.5IU; vitamin $K_3$, 4.5 mg; vitamin $B_1$, 1.5 mg; vitamin $B_2$, 9 mg; vitamin $B_6$, 1.5 mg; vitamin $B_{12}$, 15 µg; Ca-pantothenate, 22.5 mg; niacin, 105 mg; folic acid, 1.2 mg; biotin, 0.15 mg; Mn, 120 mg; Mn, 120 mg; Cu, 45 mg; Zn,120 mg; Fe, 112.5 mg; I, 0.3 mg; Mo, 0.3 mg; Co, 0.15 mg; Se, 0.15 mg.

same treatment group was pooled in a plastic bag, then 0.3 g of ceca content from each bag was put into a 15 mL vial, containing 3.7 mL mixed nutritive buffer solution (Salvador *et al.*, 1993). The incubation time, and gas sampling method were the same as experiment **I.**

Gas samples were collected using a gas-tight syringe from the sample outlet at 0 and 3 hours. Methane was analyzed using a gas chromatograph (Shimadzu, model 14 B) with an FID (Flame Ionization Detector) detector equipped with porapak Q (2 m inner diameter 1/8 inch, Supelco, PA, USA). The oven temperature was 70°C, the injection temperature was 130°C, as was the detector temperature. $N_2$ was used as the carrier gas, with a flow rate of 10 mL/min. The reference methane gas (95.5%, China petroleum Co.) was diluted to 10, 50, 100, 500 and 1000 ppm with nitrogen (98.5%) for the construction of a standard curve, and the methane concentration of each sample was then calculated according Wang *et al.* (2003). The standard curve for methane is usually linear and the $R^2$, Coefficient of Variation (CV) and minimum detecting concentration were > 0.998, < 4.7% and 0.5 ppm, respectively. The 100 ppm reference from the standard curve of previous assay was kept and used as inter assay quality control (QC). The CV for the QC has been kept as low as < 10%.

### *Statistical Analysis*

Data were analyzed by analysis of variance using the general linear model procedure. All statistical analysis was carried out using SAS software (SAS; Statistical Analysis System, 1996). The least square means were used to compare and estimate the differences among the three treatments in each experiment.

## RESULTS AND DISCUSSION

### *Methane Generation of the Intestinal Contents in Different Compartments*

A comparison of the methane generation in the intestinal contents of different compartments for mule ducklings, Muscovy ducklings and White Roman goslings at 6 weeks of age is presented in Table 3. First of all, Table 3 highlight that with the exception of goose rectum, caecum is the only site which produce methane from microbial fermentation, thus, the interaction within organs is not likely to exist. The methane generation of the cecal contents is the greatest among the various intestine compartments in each species ($p < 0.05$). The methane generation of the cecal contents or colon and rectum in goslings and mule ducklings was greater than that for Muscovy ducklings ($p < 0.05$); however, there is no significant difference in methane generation between the mule ducklings and goslings. The methane generation rate related to the duodenum, jejunum and ileum contents in birds was too low to be detected. The colon and rectum contents may have had the ability to produce methane in White Roman goslings, but this was not found in mule and Muscovy ducklings.

The above finding is in agreement with the results in Chen *et al.* (2003a),which show that the ceca is the main fermentation area in goslings, since caecectomy resulted in a 90–92% reduction of methane generation, and thus the

**Table 3.** A comparison of methane generation in the intestinal contents of different compartments among Mule ducklings, Muscovy ducklings and White Roman goslings at 6 weeks of age.

| Intestine compartments | Mule duckling | Muscovy duckling | White Roman gosling |
|---|---|---|---|
| | | ------μg/g/4 hr ------ | |
| Duodenum | ND | ND | ND |
| Jejunum | ND | ND | ND |
| Ileum | ND | ND | ND |
| Caecum | $39.32 \pm 8.46^{b}$ | $1.04 \pm 0.09^{a}$ | $43.76 \pm 24.14^{b}$ |
| Colon and rectum | ND | ND | $24.09 \pm 5.72$ |

ND: Not detected.
$^{a–b}$ Means within the same row with different superscripts differ significantly ($p < 0.05$).

cecal contents has a considerable amount of methanogenic bacterium. Therefore, cecal is the major site of methane generation in intestines of both geese and ducks.

The mean of value of the methane generation by the cecal contents with 4 hrs incubation increased 15.3 times in Muscovy ducklings when comparing the results of experiments I and II. This indicates that age is a factor in methane generation for Muscovy ducklings when they are 6–8 weeks of age, although this phenomenon is not found with mule ducklings and goslings, which have similar values of cecal content methane generation in both experiments. This finding is consistent with the results of comparing the methane generation of cecal contents with 3 hrs incubation in experiments II and IV (see Table 4 and Fig. 1).

***The Cumulative Methane Generation in Cecal Contents***

A comparison of the cumulative methane generation in the cecal contents of mule ducklings, Muscovy ducklings and White Roman goslings at 8 weeks of age for different incubation times is shown Table 4. It shows that the increases of the methane generation rate in the cecal contents are largest at 0.5–1 hr, 0–0.5 hr and 0–0.5 hr incubation time in mule ducks, Muscovy and White Roman goslings, respectively. During these incubation times, the methane generation in cecal contents increased 19.3, 4.1 and 6.3 times for mule ducklings, Muscovy ducklings and White Roman goslings, respectively, compared to the concentration at the initial incubation time. However, the cumulative methane generation in the cecal contents of Muscovy ducklings with 3 and 4 hrs incubation is the lowest among the three species ($p < 0.05$).

A comparison of methane generation among mule ducklings, Muscovy ducklings and White Roman goslings at 4 months of age is shown Fig. 1. The methane generation *in vivo* of 4-month-old birds follows the order of Muscovy ducklings > mule ducklings > White Roman goslings ($p < 0.05$).

In experiment II, The increase in methane generation of the cecal contents was greatest at 0.5–1 hr, 0–0.5 hr and 0–0.5 hr incubation times in mule ducklings, Muscovy ducklings and White Roman goslings at 8 weeks of age, respectively. This result suggests that the substrate in caecum was fermented into methane quickly at the first hour after arriving the cecaem. Thisis similar to that in Chen (2003), which showed that the increase in methane generation of cecal contents is the greatest during 0–1 hr incubation time in geese. However, the increase in methane generation rate was decreased over time, and this is probably because the substrate in the cecal contents is exhausted, or acetic acid was accumulated in the fermentative generation process, resulting in lower levels of activity in the methanogenic bacterium. Other studies show that acetic acid is major volatile fatty acid in the ceca of goslings (Chen *et al.*, 1992), and that acetic acid suppresses methane generation (Beijer, 1952; Armstrong and Blaxter, 1965; Blaxter and Czerkawski 1966; Chen *et al.*, 2009). Therefore, these factors may inhibit the methane generation rate in the cecal contents when the incubation time lasts for 1 hour. The length of colon and rectum in poultry are very short (about 10 cm), and the

**Table 4.** A comparison of cumulative methane generation in cecal contents among Mule ducklings, Muscovy ducklings and White Roman goslings at 8 weeks of age for different incubation time.

| *In vitro* incubation time | Mule duckling | Muscovy duckling | White Roman gosling |
|---|---|---|---|
| | | -------μg/g------ | |
| 0 hr | $0.45 \pm 1.31$ | $0.44 \pm 0.1.35$ | $1.24 \pm 1.54$ |
| 0.5 hr | $0.67 \pm 0.48$ | $1.82 \pm 0.63$ | $7.79 \pm 1.73$ |
| 1 hr | $12.92 \pm 3.82$ | $2.31 \pm 0.58$ | $8.76 \pm 0.04$ |
| 2 hr | $18.35 \pm 4.26$ | $2.42 \pm 1.14$ | $9.81 \pm 10.71$ |
| 3 hr | $32.06 \pm 26.59^{b}$ | $5.43 \pm 5.24^{a}$ | $37.79 \pm 3.79^{b}$ |
| 4 hr | $41.37 \pm 0.95^{b}$ | $15.86 \pm 12.16^{a}$ | $39.24 \pm 3.43^{b}$ |
| Orthogonal contrast | | | |
| Linear | * | * | * |
| Quadratic | NS | NS | NS |

$^{a–b}$ Means within the same row with different superscripts differ significantly ($p < 0.05$).
* $p < 0.05$; NS: Not significant.

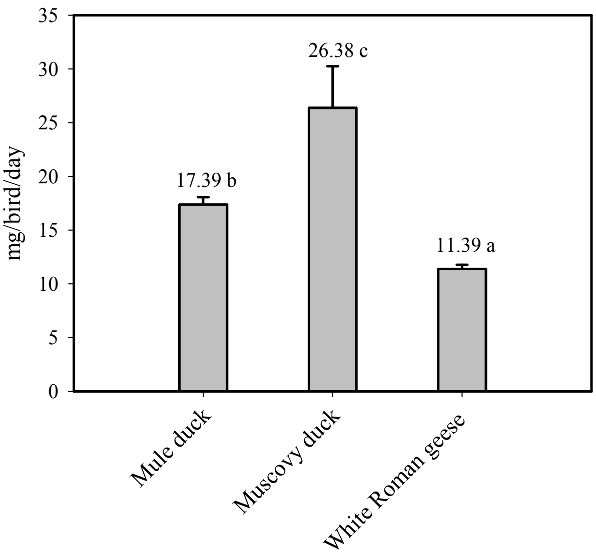


**Fig. 1.** The comparison of methane generation *in vivo* among Mule ducklings, Muscovy ducklings and White Roman goslings at 4 months of age.
[a–c] Means within the same row with different superscripts differ significantly ($p < 0.05$).

digesta pass through the large intestine in a very short time. In addition, the colon microbial digestion is not well developed; therefore, post cecal methane fermentation is unlikely to occur. The ceca in goslings is the main fermentation area since caecetomy resulted in a 90–92% reduction of methane generation (Chen *et al.*, 2003). Moreover, digesta entering the cecum in the first hour produced large quantity of methane (Chen *et al.*, 2009).

Based on the methane generation of cecal contents *in vitro* for 3 or 4 hrs incubation in experiments I, II and Ⅳ, Muscovy ducklings have the lowest mean value of methane concentration. This suggests that Muscovy ducklings may have poor fermentative capacity, due to having fewer methanogenic bacterium and les formic acid substrate in the cecal contents. However, comparing the results of experiments II with III, the *in vitro* methane generation of the cecal contents of 6-week-old Muscovy ducklings had the lowest mean value among the three species, while the *in vivo* methane concentration of the cecal contents in 4-month-old Muscovy ducks had the highest mean value of methane generation rate among the three species in experiment III. This may be because of the digestive tract physiology in these species, and specifically for the following reasons:

1. Cecal contents weight: The growth of Muscovy drakes at 8 weeks of age is slower than that of ganders, but the growth of the former lasts until 12 weeks of age (Scott and Dean, 1991; Chen *et al.*, 2003), while mule ducks grow slowly after 10 weeks of age (Chen and Roan, 2005). After 8 weeks of age, the small intestinal digesta of Muscovy ducks may enter into the ceca as they get older, while the reverse is true for White Roman geese and mule ducks. Moreover, because the amount of abdominal fat and mesentery fat increase rapidly with age in geese (Chen *et al.*, 2003b; Chen and Hsu, 2004), and this reduces the amount of space of in the abdominal cavity, thus reducing feed intake, so the amount of digesta that enters into the ceca also decreases. Therefore, Muscovy ducks may have the largest cecal content weight among the three species.
2. Cecal content composition: It may be supposed that the nutrients metabolized in the intestinal tract of birds changes as they get older, and this leads to greater amounts of the fermentation substrate being converted into formic acid in the ceca. Therefore, Muscovy ducks had greater methane generation in the ceca, and this is similar to the findings in Tsukahara and Ushida (2000) that the methane generation of chicks is affected by diet composition.
3. Microbial clone: Methanogenic bacterial growth may increase due to changes in the composition of cecal contents with age in Muscovy ducks.
4. Empty times and quantity in ceca: The ceca empty times in birds depends on the species, e.g., The ceca of turkey empty only once every 24 hours (Duke *et al.*, 1984), while with ptarmigan the ceca digesta are emptied once every 8.5 hours and 56% of the cecal content was emptied each time (Gasaway and Holleman, 1975). Chen (2003) states that geese empty the large cecal feces 1–2 times a day, and a small amount is emptied every 4–6 hours. The flow dynamics of digesta affect its composition in ceca (Stevens and Hume, 1995). Therefore, it is suggested that the empty times is less and quantity of ceca is higher in Muscovy ducks that in the other two species examined in this work, and this may be the reason why, *in vivo*, these birds have more complete fermentation than mule ducks and geese.
5. Others: The method used to determine methane generation also affects the experimental results. e.g., in experiments I and II, an *in vitro* method and cecal content basis approach were used to determine methane generation; however, in experiment III, the *in vivo* method and bird basis approach were used to determine methane generation. Moreover, there may also be other factors that influence the results, and further research is required to examine this issue.

### *The Effect of Alfalfa Meal on in Vitro Methane Generation of Cecal Contents*

A comparison of methane generation among mule ducklings, Muscovy ducklings and White Roman goslings at 7 weeks of age is presented in Fig. 2. The cumulative methane generation of the cecal contents in 4 hours reduced significantly when the birds were fed grower diet supplement with 30% alfalfa meal, with a generation of 12.0 µg/g as compared to 36.1 µg/g with the grower diet in mule ducklings, 5.1 µg/g as compared to 13.9 µg/g with the grower diet in Muscovy ducklings, and 34.2 µg/g as compared to 1.54 µg/g with the grower diet in goslings ($p < 0.05$), and the reductions in methane generation for mule ducklings, Muscovy ducklings and geese were 66.8%, 63.3% and 95.5%, respectively. The methane generation of the cecal contents of the goslings was thus the lowest among the three species fed a diet containing alfalfa meal ($p < 0.05$).

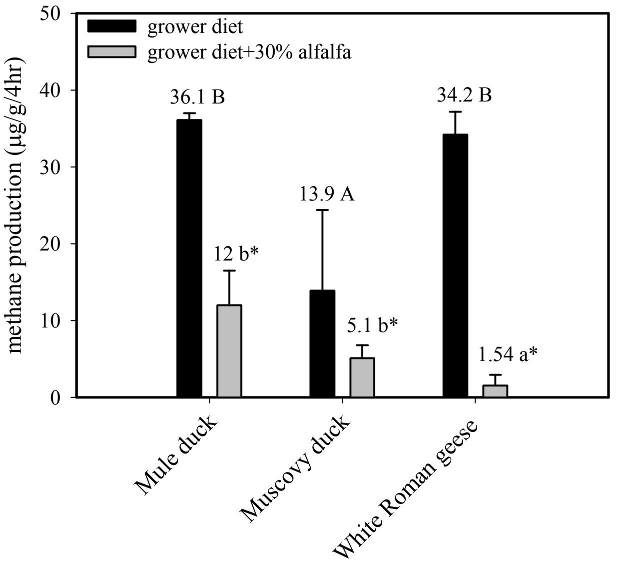


**Fig. 2.** Effects alfalfa additive on the methane generation of the cecal contents *in vitro* among Mule ducklings, Muscovy ducklings and White Roman goslings at 7 weeks of age.
$^{A,B}$ Means within bird fed grower diet with different superscripts differ significantly ($p < 0.05$).
$^{a,b}$ Means within bird fed grower diet + 30% alfalfa meal with different superscripts differ significantly ($p < 0.05$).
* Means between two diets differ significantly for the same species ($p < 0.05$).

The methane generation of cecal contents in birds fed grower diet supplemented with 30% alfalfa meal is significantly lower than that of birds fed with grower diet. This indicates that mule ducklings, Muscovy ducklings and White Roman goslings have similar cecal fermentation physiologies, i.e., alfalfa meal affects the methane generation of the cecal contents in waterfowl. The reduction in the methane concentration of the cecal contents in goslings was the greatest among the three species, and this suggests that the methanogenic bacteria in the cecal contents of goslings are sensitive to alfalfa meal.

The reason why alfalfa meal inhibited cecal methanogenesis in birds. This might be related to saponin. Pond (1984) notes that the saponin contents are 1.46% in selected alfalfa meal and 2.37% in unselected alfalfa meal. Wang *et al.* (1998) demonstrate that the rich saponin content of *Yucca schidigera* extractat 5 mg/mL reduced total bacteria numbers in the rumen simulation technique by more than 55%. In addition, evidence of saponin affecting methanogenesis microbes is also given in Wina *et al.* (2005), which reports that methanogens decreased with the use of 4 mg/mL saponin containing methanol extract of *Sapindus rarak*. Hess *et al.* (2005) noted that a diet containing 0.012% saponin from *Sapindus saponaria* decreased methanogenesis by 29% in defaunated rumen fluid versus 14% in faunated rumen fluid. In this study, the saponin content in the grower diet supplemented with 30% alfalfa meal was 0.55%, as calculated on a 2.37% saponin basis in unselected alfalfa. Therefore, it is suggested that the saponin in alfalfa meal may be the major factor responsible for reducing *in vitro* methane generation in the cecal contents in birds.

The reason why the methane generation rate of the cecal contents of gosling fed with alfalfa meal was the lowest among the three species examined in this work is unknown. However, goose may have a better capability to digest alfalfa than ducks, and thus more saponin was dissociated from alfalfa to suppress methanogenic bacteria. Moreover, whether methane generation is related to saponin dissociation from alfalfa remains unclear, and more work is needed to examine this issue.

## CONCLUSIONS

This study was to compare the methane generation in different compartments of the intestine at different growth stages. In addition, the alfalfa was tested as an additive to lower the methanol generation in the three species. In this framework, the following conclusions can be drived:

- *In vitro*, the cecum is the major methane generation site among the various intestine compartments in each species.
- The increases of the methane generation rate in the cecal contents are largest at 0.5–1 hr, 0–0.5 hr and 0–0.5 hr incubation time in mule ducks, Muscovy and White Roman goslings, respectively. It shows that during these incubation times, the methane generation in cecal contents increased 19.3, 4.1 and 6.3 times for mule ducklings, Muscovy ducklings and White Roman goslings, respectively, compared to the concentration at the initial incubation time.
- Both Age and the method of measurement (*in vitro* vs. *in vivo*) affect the methane generation results for the three species.
- The cumulative methane generation of the cecal contents in mule ducklings, Muscovy ducklings, and goslings were 36.1, 13.9, and 34.2 μg/g/4 h in goslings.
- By using 30% alfalfa meal feed, the cumulative methane generation of the cecal contents for mule ducklings, Muscovy ducklings and geese reduce by 66.8%, 63.3% and 95.5%, respectively. Consensually, the use of alfalfa as a feed is highly potential with regard to decreasing greenhouse gases emission for waterfowl.


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## REFERENCES


Annison, E.F., Kill, K.J. and Kenworthy, R. (1968). Volatile Fatty Acids in the Digestive Tract of the Fowl. *Br. J.*

*Nutr.* 22: 207–216.

Armstrong, D.G. and Blaxter, K.L. (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.

Blaxter, K.L. and Czerkawski, J. (1966). Modifications of the Methane Production of the Sheep by Supplementation of Its Diet. *J. Sci. Food Agric.* 17: 417–421.

Chen, Y.H., Hsu, J.C. and Yu, B. (1992). Effects of Dietary Fiber Levels on Growth Performance, Intestinal Fermentation and Cellulase Activity of Goslings. *J. Chin. Soc. Anim. Sci.* 21: 15–28 (in Chinese).

Chen, Y.H. (2003). Studies on the Cecal Morphology, Development and Methane Production of White Roman Geese, Ph.D. Dissertation, National Chung Hsing University, Taichung, Taiwan.

Chen, Y.H., Wang, S.Y. and Hsu, J.C. (2003a). Effects of Caecectomy on Body Weight Gain, Intestinal Characteristics and Enteric Gas Production in Goslings. *Asian Australas J. Anim. Sci.* 16: 1030–1034.

Chen, Y.H., Hsu, J.C., Shih, B.L., Lio, D.C. and Chen, M.T. (2003b). A Study on the Optimal Marketing Age of Geese. *J. Chin. Soc. Anim. Sci.* 32: 111–121 (in Chinese).

Chen, Y.H. and Hsu, J.C. (2004). The Development of Digestive Tract in White Roman and Chinese Geese at Finishing Period. *Tunghai J.* (College of Agriculture) 45: 1–7.

Chen, Y.H. and Roan, S.W. (2005). Growth Curve of white Mule Ducks. *J. Chin. Soc. Anim. Sci.* 34: 31–37 (in Chinese).

Chen, Y.H., Wang, S.Y. and Hsu, J.C. (2009). *In vivo* Methane Production from Formic and Acetic Acids in the Gastrointestinal Tract of White Roman Geese. *Asian Australas J. Anim. Sci.* 22: 1043–1047.

Clemens, E.T., Stevenes, C.E. and Southworth, M. (1975). Site of Organic Acid Production and Pattern of Digesta Movement in Gastrointestinal Tract of Geese. *J. Nutr.* 105: 1341–1350.

Duke, G.E., Eccleston, E., Kirywood, S., Louis, C.F. and Bedbury, H.P. (1984). Cellulose Digestion by Domestic Turkeys Fed Low and High Fiber Diets. *J. Nutr.* 114: 95–102.

Gasaway, W.C., Holleman, D.F. and White, R.C. (1975). Flow of Digesta in the Intestine and Cecum of the Rock Ptarmigan. *Conder* 77: 467–474.

Gasaway, W.C. (1976). Cellulose Digestion and Metabolism by Captive Rock Ptarmigan. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* 54: 179–182.

Hess, H.D., Kreuzer, M., Díaz, T.E., Lascano, C.E., Carulla, J.E. and Soliva, C.R. (2003). Machmüller, Andrea Saponin Rich Tropical Fruits Affect Fermentation and Methanogenesis in Faunated and Defaunated Rumen Fluid. *Anim. Feed Sci. Technol.* 109: 79–94.

Hetzel, D.J.S. (1985). Domestic Ducks: An Historical Perspective, In *Duck Production Science and World Practice*, Farrell, D.J. and Stalieton, P. (Ed.), New England University Press, Armidale, N. S. W. p.1–5.

Heywang, B.W. and Bird, H.R. (1954). The Effect of Alfalfa Saponin on the Growth, Diet Consumption and Efficiency of Diet Utilization of Chicks. *Poultr. Sci.* 33: 239–241.

Holtshausen L., Chaves, A.V., Bwauchemin, K.A., McGill, S.M., McAllister, T.A., Odongo, N.E., Cheeke, P.R. and Benchaar, C. (2009). Feeding Saponin-containing Yucca Schidigera and Quillaja Saponaria to Decrease Enteric Methane Production in Dairy Cows. *J. Dairy Sci.* 92: 2809–2821.

Hu, W.L., Lin, J.X., Ye, J.A., Wu, Y.M. and Guo, Y.Q. (2005). Effect of Tea Saponin on Rumen Fermentation in Vitro. *Anim. Feed Sci. Technol.* 120: 333–339.

IPCC (Intergovermental Panel on Climate Change) (2001). *Climate Change 2001*, The Scientific Basis, Cambrige University Press, Cambrige, UK.

Jasim, M.R., Matjafri, M.Z. and Lim, H.S. (2012). Methane Interannual Distribution over Peninsular Malaysia from Atmospheric Infrared Sounder Data: 2003-2009. *Aerosol Air Qual. Res.* 12: 1459–1466.

McBee, R.H. (1969). Cecal Fermentation in the Willow Ptarmigan. *Conder* 71: 54–58.

Pond, W.G. and Maner, J.H. (1984). *Swine Production and Nutrition*, AVI, Westport, Conn. p. 501–501.

SAS (1988). SAS User's Guide: Statistics, Releases 6.03 ed. SAS Inst., Inc., Cary, NC.

Scott, M.L. and Dean, W.F. (1991). *Nutrition and Management of Ducks*, M.L. Scott of Ithaca Publisher, Ithaca, New York, p. 40.

Stevens, C.E. and Hume, I.D. (1995). Comparative Physiology of the Vertebrate Digestive System, Cambrige University, New York, USA, p. 21–147.

Tsukahara, T. and Ushida, K. (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. A: Mol. Integr. Physiol.* 127: 139–146.

Van Kessell, J.A.S. and Russell, J.B. (1996). The Effect of pH on Ruminal Methanogenesis. *FEMS Microbiol. Ecol.* 20: 205–210.

Wang, S.Y., Shieh, S.W., Wang, S.H. and Chen, Y.H. (2003). Assessment of Enteric Fermentation Emission Factors of Greenhouse Gases in Goose Utilizing a Respiratory Chamber. *J. Chin. Soc. Anim. Sci.* 43: 43–50 (in Chinese).

Wang, Y., McAllister, T.A., Newbold, C.J., Rode, L.M., Cheeke, P.R. and Cheng, K.J. (1998). Effects of *Yucca schidigera* Extract on Fermentation and Degradation of Steroidal Saponin in the Rumen Simulation Technique (RUSITEC). *Anim. Feed Sci. Technol.* 74: 143–153.

Wina, E., Muetzel, S., Hoffmann, E., Makkar, H.P.S. and Becker, K. (2005). Saponins Containing Methanol Extract of *Sapindus rarak* Affect Microbial Fermentation, Microbial Activity and Microbial Community Structure in vitro. *Anim. Feed Sci. Technol.* 105: 163–184.

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