

GREEN HOUSE GAS CONTROL AND AGRICULTURAL BIOMASS FOR SUSTAINABLE ANIMAL AGRICULTURE IN DEVELOPING COUNTRIES

J. TAKAHASHI

Graduate School of Animal Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan 080-8555

(Makalah diterima 23 Maret 2010 – Revisi 6 Mei 2010)

ABSTRACT

Important green house gases (GHG) attributed to animal agriculture are methane (CH₄) and nitrous oxide (N₂O), though carbon dioxide (CO₂) contributes almost half of total greenhouse effect. Rumen CH₄ production in an enteric fermentation can be accounted as the biggest anthropogenic source. Some of prebiotics and probiotics have been innovated to mitigate rumen CH₄ emission. The possible use of agricultural biomass consisted of non-edible parts of crop plants such as cellulose and hemicellulose and animal wastes was proposed as a renewable energy and nitrogen sources. The ammonia stripping from digested slurry of animal manure in biogas plant applied three options of nitrogen recycling to mitigate nitrous oxide emission. In the first option of the ammonia stripping, the effect of ammonolysis on feed value of cellulose biomass was evaluated on digestibility, energy metabolism and protein utilization. Saccharification of the NH₃ treated cellulose biomass was confirmed in strictly anaerobic incubation with rumen cellulolytic bacteria, *Ruminococcus flavefaciens*, to produce bio-ethanol as the second option of ammonia stripping. In an attempt of NH₃ fuel cell, the reformed hydrogen from the NH₃ stripped from 20 liter of digested slurry in thermophilic biogas plant could generate 0.12 W electricity with proton exchange membrane fuel cell (PEM) as the third option.

Key words: GHG, rumen, methane, probiotics, ammonia stripping, biomass

ABSTRAK

PENGATURAN GAS RUMAH KACA DAN BIOMASA PERTANIAN UNTUK USAHA PETERNAKAN BERKELANJUTAN DI NEGARA BERKEMBANG

Gas rumah kaca (GRK) yang penting dan berkaitan dengan peternakan adalah metana (CH₄) dan nitrogen oksida (N₂O) meskipun karbon dioksida (CO₂) menyebabkan hampir 50% dari efek rumah kaca. Metana yang berasal dari pencernaan ruminansia merupakan sumber antropogenik yang utama. Prebiotik dan probiotik yang dihasilkan telah mampu menekan emisi metana dari rumen. Pemanfaatan biomasa pertanian yang berasal dari komponen yang tidak dapat dimakan seperti selulosa dan hemiselulosa serta limbah ternak disarankan untuk digunakan sebagai sumber energi dan nitrogen terbarukan. Penjaringan amonia dari limbah ternak dalam usaha pembuatan biogas dilakukan dalam tiga alternatif daur ulang nitrogen untuk mengurangi emisi nitrogen oksida. Pada alternatif pertama, telah diamati pengaruh amonialisasi terhadap nilai biomasa selulosa dari aspek pencernaan, metabolisme energi dan pemanfaatan protein. Sakarifikasi selulosa melalui perlakuan NH₃ dalam inkubasi anaerob menggunakan mikroba selulolitik rumen, *Ruminococcus flavefaciens*, menghasilkan bio-etanol sebagai alternatif kedua dari penjaringan amonia. Pada percobaan baterai berbahan bakar NH₃, pembentukan hidrogen dari NH₃ yang dijarah dari 20 liter limbah tercerna dalam pembuatan biogas termofilik dapat menghasilkan 0,12 W listrik menggunakan baterai membran tukar proton (PEM = *proton exchange membrane*) sebagai alternatif ketiga.

Kata kunci: GRK, metana, rumen, probiotik, penjaringan amonia, biomasa

INTRODUCTION

The mitigation of anthropogenic six GHG such as carbon dioxide, methane, nitrous oxide, sulphur hexafluoride, hydrofluorocarbons and perfluorocarbons have been established as legally binding commitments in The Kyoto Protocol (IPCC, 1996). Important GHG attributed to animal agriculture are methane (CH₄) and nitrous oxide (N₂O). Rumen fermentation of ruminant livestock and anaerobic fermentation of agricultural

organic waste including animal manures are major contributors of CH₄ emission as anthropogenic sources (MOSS, 1993).

To abate the GHG emission, the development of mitigation methods of rumen CH₄ is the most significant issue in the world ruminant livestock production (VAN NEVEL and DEMEYER, 1996). The prompt increase of atmospheric N₂O since last century is closely related to abrupt expansion of human and animal population after an innovation of Haber-Bosch

process. Severe environmental pollutions were caused at the same time though the reactive nitrogen withdrawn from atmosphere as stable paired nitrogen brought about prosperous food production. To secure food production, preventing environmental catalyses by global warming, sustainable development of animal agriculture should be sought in not only developed but also developing countries as an alternative way. Inventories of emitters and their abatements are accurately assessed in both GHG to develop “clean developing mechanism (CDM)” in Kyoto Mechanism. The CDM might give highly economical and environmental incentives for the implementation in developing countries. The key element of these recyclings must be low-input for sustainable animal agriculture in developing countries. Carbon and nitrogen recycling in the agricultural biomass as renewable energy and nitrogen resources might contribute mitigation of CH₄ and N₂O (TAKAHASHI *et al.*, 2003; 2004; TAKAHASHI and UEMURA, 2009).

The present paper deals with perspective on GHG control and possible uses of biomass towards sustainable animal agriculture.

MITIGATION OF RUMEN METHANE EMISSION WITH PREBIOTICS AND PROBIOTICS

In the rumen, metabolic H₂ is produced during the anaerobic fermentation of glucose. This H₂ can be used during the synthesis of volatile fatty acids and microbial organic matter. The excess H₂ from NADH is

eliminated primarily by the formation of CH₄ by methanogens, which are microorganisms from the *Archaea* group that are normally found in the rumen ecosystem (Baker, 1999). The stoichiometric balance of VFA, CO₂ and CH₄ indicates that acetate and butyrate promote CH₄ production whereas propionate formation conserves H₂, thereby reducing CH₄ production (WOLIN and MILLER, 1988). Therefore, a strategy to mitigate ruminal CH₄ emission is to promote alternative metabolic pathway to dispose of the reducing power, competing with methanogenesis for H₂ uptake. The administration of nitrate remarkably suppressed ruminal methanogenesis (TAKAHASHI and YOUNG, 1991; 1992).

Figure 1 shows that the formation of toxic nitrite reduced from nitrate is successfully prevented by L-cysteine (TAKAHASHI and YOUNG, 1991, 1992; TAKAHASHI *et al.*, 1989, 1998, 2000, 2002), *i.e.* the effective mitigation of ruminal CH₄ emission is safely achieved by simultaneous administration of nitrate and L-cysteine without nitrite intoxication (TAKAHASHI, 2001). Furthermore, *Escherichia coli* modified genetically was developed in an attempt to promote nitrite reduction abating ruminal methanogenesis (SAR *et al.*, 2004a; 2005a; 2005b; 2005c) (Figure 2).

Rumen manipulation with ionophores such as monensin has been reported to abate rumen methanogenesis (MWENYA *et al.*, 2005). However, there is an increasing interest in exploiting prebiotics and probiotics as natural feed additives to solve problems in animal nutrition and livestock production as alternatives of the antibiotics due to concerns about

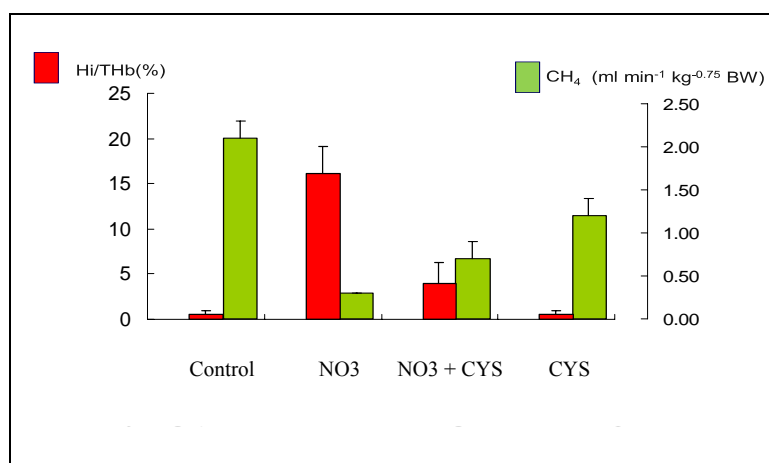
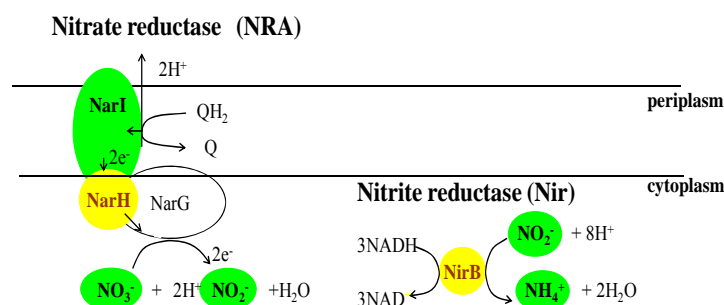


Figure 1. The suppressing effect of nitrate (1.3 g NaNO₃ kg^{-0.75} body weight) on methane emission and prophylactic effect of L-cysteine (0.21 g S kg^{-0.75} body weight) on nitrate induced methemoglobinemia in sheep

1. Wild-type *E. coli* W3110



2. Construction of *E. coli* nir-Ptac by replacement of self-promoter (*nir*) in *E. coli* W3110 by *tac* promoter (Ptac) (Ajinomoto Co. Inc., Kawasaki, Japan)

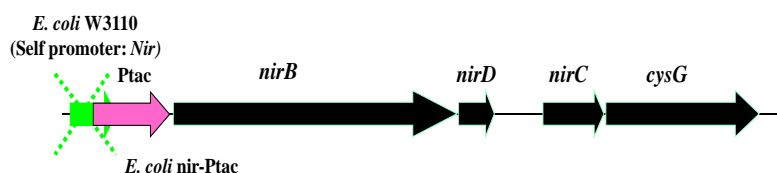


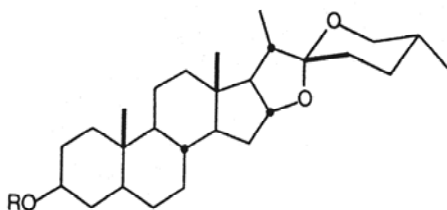
Figure 2. Wild-type *E. coli* W3110 and the construction of *E. coli* nir-Ptac

incidences of resistant bacteria and environmental pollution by the excreted active-antibacterial substances (MWENYA *et al.*, 2006). Nisin and saponin-containing extracts of *Yucca schidigera* and *Quillaja saponaria* have been categorized as 'generally recognized as safe (GRAS)' for human consumption by US Food and Drug Administration. Nisin produced by *Lactococcus lactis* subsp. *lactis*, antimicrobial activity against spectrum of Gram-positive bacteria is characterized bacteriocin and performed mitigating effect on ruminal methane emission (MWENYA *et al.*, 2004a; SANTOSO *et al.*, 2004b; SAR *et al.*, 2006). Saponins are natural detergents found in variety of plants. *Yucca* saponins have a steroidal nucleus, whereas *Quillaja* saponins are triterpenoid in structure (Figure 3). Supplementation of saponin-rich plant extracts decreased ruminal protozoa counts and decreased methanogenesis accompanied by decrease in the ruminal acetate/propionate (A/P) ratio *in vitro* and *in vivo* (WALLES *et al.*, 1994; WANG *et al.*, 2000, TAKAHASHI *et al.*, 2000; SANTOSO *et al.*, 2004a; MWENYA *et al.*, 2004a; PEN *et al.*, 2006). However, PEN *et al.* (2007; 2008) showed in recent detailed examination that *Q. saponaria* had no effect on ruminal

methanogenesis and A/P ratio, although it suppressed protozoa number.

Galacto-oligosaccharides (GOS) are non-digestible carbohydrates in nonruminants and have a long history of research as a prebiotics food ingredient. GOS are resistant to gastrointestinal enzymes, but are selectively utilized *Bifidobacteria* (SAKO *et al.*, 1999). In the rumen, *Bifidobacteria* and *Lactobacillus* species utilize fructose, galactose, glucose and starch as substrates to produce lactate and acetate. Lactate is intermediate compound of an acrylate pathway during propionate production in the rumen. Meanwhile, propionate production is indirect competition with methanogens for available hydrogen. As *Bifidobacteria* and *Lactobacillus* species in the rumen can utilize GOS and produce more lactate, ruminal methanogenesis have been suppressed by GOS with or without direct-fed microbe yeasts and lactic acid bacteria (GAMO, 2001; MWENYA *et al.*, 2004b; 2004c; 2004d; 2005; SANTOSO, 2004a; SAR *et al.*, 2002; 2004b; 2004c; TAKAHASHI *et al.*, 2002; 2003). However, the efficacy of GOS with the probiotics on different diets and animal species remains to be elucidated.

Yucca schidigera
(Steroidal saponins)



Quillaja saponaria
(triterpenoid saponins)

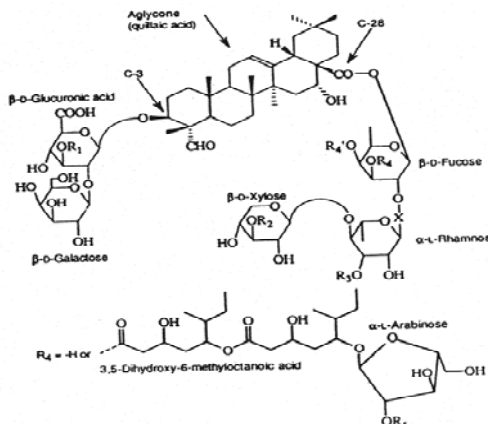


Figure 3. Chemical structure of *Yucca schidigera* and *Quillaja saponaria*

Creation of renewable energy (biogas) from anaerobic fermentation (biogas plant) of animal manures and the innovative reuse of the digested slurry

The increased emissions of CH₄ and N₂O from decomposing unmanaged and bio-based industrial wastes along with the expansion of human activities contribute climate change as GHG. The biogas plant produce biogas including combustible CH₄ as renewable energy using unused resources like animal manures, can provide fuel, heat and electricity (TAKAHASHI *et al.*, 2004; UMETSU *et al.*, 2005, 2006; KOMIYAMA *et al.*, 2006), and minimize the impact on the environment thus reducing the amount of pollutants discharged. Biogas system and its application have been expanded in APEC member economies as a mitigation strategy with an economical incentive

(APEC, 2009). The conventional biogas system based on anaerobic fermentation of the organic wastes, however, is not a nitrogen recycling but a carbon recycling. Therefore, isometric fertilization of the digested slurry after anaerobic fermentation may not be a solution of current issue on excess nitrogen abatement, although nitrous oxide emission is almost completely suppressed during anaerobic fermentation (unpublished data). It causes not only methane emission, but also nitrate leaching and N₂O emission from soil (TAKAHASHI, 2006).

The introduction of ammonia stripping from digested slurry of thermophilic biogas plant might be a solution to reduce total nitrogen content of the slurry as a liquid fertilizer containing suitable nitrogen and eventually can contribute the mitigation of N₂O emission as a new concept of biogas system (Figure 4). Furthermore, the stripped ammonia can be put to

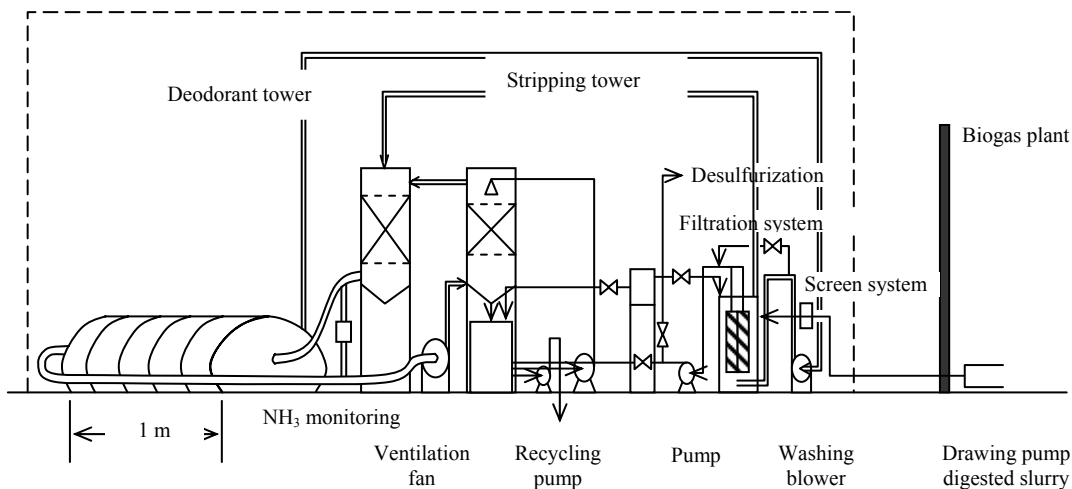
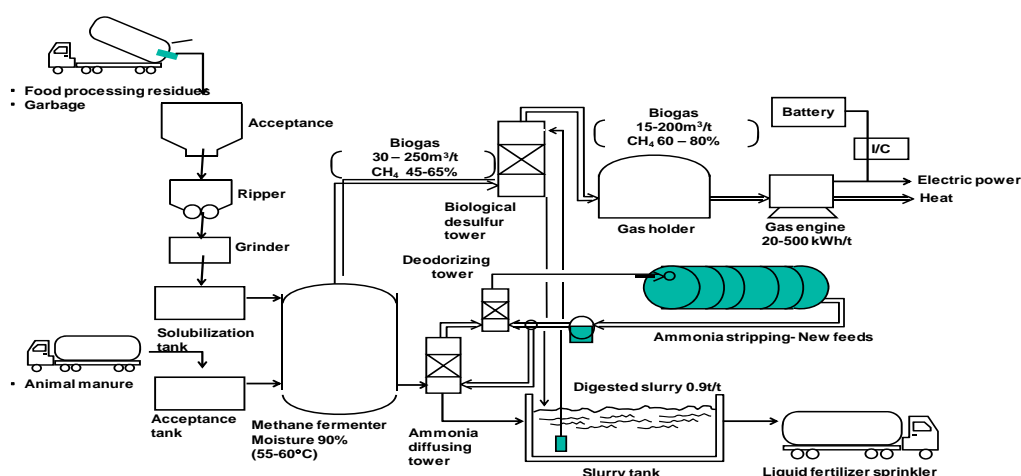


Figure 4. Ammonia stripping apparatus



I **Figure 5.** Biogas system addressed biomass to local society

practical use as a low-input and renewable nitrogen resource without energy supply from outside, because abundant amount of organic wastes exist in developing countries and the energy required for ammonia stripping can be supplied from biogas plant attached to the ammonia stripping apparatus. Figure 5 shows biogas system addressed biomass to local society.

The following three options have been examined for future nitrogen recycling:

1. Production of high quality feed from cellulose biomass in agricultural waste with ammonia stripping process from digested slurry of biogas plant (TAKAHASHI, 2006; 2007).
2. Saccharification of soft cellulose biomass to create bio-ethanol and hydrogen using ammonolysis by stripped ammonia from effluent and hydrolysis of rumen bacteria (unpublished).
3. Ammonia fuel cell with ammonia stripping from digested slurry (TAKAHASHI and UEMURA, 2009).

CONCLUSION

Controlling the GHG emission from agricultural biomass for sustainable animal production in the developing countries can be conducted by supplementation of feed with probiotics or prebiotics, ammonia stripping from digested slurry of biogas plant; saccharification of soft cellulose biomass to create bio-ethanol and hydrogen; and to develop ammonia fuel cell with ammonia stripping from digested slurry.

REFERENCES

- APEC. 2009. Proc. of International Workshop On Sustainable Green Livestock Agriculture Confronting Climate Change in APEC Member Economies. RDA, Korea pp. 1 – 265.
- BAKER, S.K. 1999. Rumen methanogens, and inhibition of methanogenesis. *Aust. J. Agric. Res.* 50: 1293 – 1298.
- GAMO, Y., M. MII, X.G. ZHOU, C. SAR, B. SANTOSO, I. ARAI, K. KIMURA and J. TAKAHASHI. 2001. Effects of lactic acid bacteria, yeasts and galactooligosaccharide supplementation on *in vitro* rumen methane production. Proc. of the 1st International Conference on Greenhouse Gases and Animal Agriculture (GGAA). Obihiro, Japan, 7 – 11 Nov. 2001, Obihiro, Hokkaido, Japan pp. 371 – 374.
- IPCC. 1996. Revised 1996 IPCC Guidelines for National Greenhouse Gas Inventories. IPCC-NGGIP.
- KOMIYAMA, M., K. UMETSU and J. TAKAHASHI. 2006. Biogas as a reproducible energy source: Its steam reforming for electricity generation and for farm machine fuel. *In: Greenhouse Gases and Animal Agriculture: An Update.* SOLIVA, R.C., J. TAKAHASHI and M. KREUZER (Eds.) Elsevier Sci. pp. 234 – 237.
- MOSS, A.R. 1993. Methane: Global Warming and Production by Animals. Canterbury. Chalcombe Publications.
- MWENYA, B., B. SANTOSO, C. SAR, B. PEN, R. MORIKAWA, K. TAKAURA, K. UMETSU, K. KIMURA and J. TAKAHASHI. 2004c. Effects of yeast culture and galactooligosaccharides on ruminal fermentation in Holstein cows. *J. Dairy Sci.* 88: 1404 – 1412.

- MWENYA, B., B. SATONSO, C. SAR, Y. GAMO, T. KOBAYASHI, I. ARAI and J. TAKAHASHI. 2004d. Effects of including β 1-4 galacto-oligosaccharides, lactic acid bacteria or yeast culture on methanogenesis as well as energy and nitrogen metabolism in sheep. *Anim. Feed. Sci. Technol.* 115: 313 – 326.
- MWENYA, B., C. SAR, B. PEN, R. MORIKAWA, K. TAKAURA, S. KOGAWA, K. KIMURA, K. UMETSU and J. TAKAHASHI. 2006. Effects of feed additives on ruminal methanogenesis and anaerobic fermentation of manure in cows and steers. *In: Greenhouse Gases on Animal Agriculture update. International Congress Series.* SOLIVA, C., J. TAKAHASHI and M. KREUTZER (Eds.). Elsevier, Amsterdam 1293: 209 – 212.
- MWENYA, B., C. SAR, B. SANTOSO, T. KOBAYASHI, R. MORIKAWA, K. TAKAURA, K. UMETSU, S. KOGAWA, K. KIMURA, H. MIZUKOSHI and J. TAKAHASHI. 2005. Comparing the effects of β 1-4 galacto-oligosaccharides and L-cysteine to monensin on energy and nitrogen utilization in steers fed a very high concentrate diet. *Anim. Feed Sci. Technol.* 118: 19 – 30.
- MWENYA, B., C. SAR, Y. GAMO, T. KOBAYASHI, R. MORIKAWA, K. KIMURA, H. MIZUKOSHI and J. TAKAHASHI. 2004a. Effects of *Yucca schidigera* with or without nisin on ruminal fermentation and microbial protein synthesis in sheep fed silage- and hay- based diets. *Anim. Sci. J.* 75: 525 – 531.
- MWENYA, B., X. ZHOU, B. SANTOSO, C. SAR, Y. GAMO, T. KOBAYASHI and J. TAKAHASHI. 2004b. Effects of probiotic-vitacogen and β 1-4 galacto-oligosaccharides supplementation on methanogenesis and energy and nitrogen utilization in dairy cows. *Asian-Aust. J. Anim. Sci.* 17: 349 – 354.
- PEN, B., C. SAR, B. MWENYA, K. KUWAKI and J. TAKAHASHI. 2008. Effects of *Quillaja saponaria* extract alone or in combination with *Yucca schidigera* extract on ruminal fermentation and methanogenesis *in vitro*. *Anim. Sci. J.* 79: 193 – 199.
- PEN, B., C. SAR, B. MWENYA, K. KUWAKI, R. MORIKAWA and J. TAKAHASHI. 2006. Effects of *Yucca schidigera* and *Quillaja saponaria* extracts on *in vitro* ruminal fermentation and methane emission. *Anim. Feed Sci. Technol.* 129: 175 – 186.
- PEN, B., K. TAKAURA, S. YAMAGUCHI, R. ASA and J. TAKAHASHI. 2007. Effects of *Yucca schidigera* and *Quillaja saponaria* with or without β 1-4 galacto-oligosaccharides on ruminal fermentation, methane production and nitrogen utilization in sheep. *Anim. Feed Sci. Technol.* 138: 75 – 88.
- SAKO, T., K. MATSUMOTO and R. TANAKA. 1999. Recent progress on research and application of non-digestible galacto-oligosaccharides. *Int. Dairy J.* 9: 69 – 80.
- SANTOSO, B., B. MWENYA, C. SAR, Y. GAMO, T. KOBAYASHI, R. MORIKAWA, K. KIMURA, H. MIZUKOSHI and J. TAKAHASHI. 2004a. Effects of supplementing galacto-oligosaccharides, *Yucca schidigera* or nisin on rumen methanogenesis, nitrogen and energy metabolism in sheep. *Livest. Prod. Sci.* 91: 209 – 217.
- SANTOSO, B., B. MWENYA, C. SAR, Y. GAMO, T. KOBAYASHI, R. MORIKAWA and J. TAKAHASHI. 2004b. Effect of *Yucca schidigera* with or without nisin on ruminal fermentation and microbial protein synthesis in sheep fed silage- and hay-based diets. *Anim. Sci. J.* 75: 525 – 531.
- SAR, C., B. SANTOSO, B. MWENYA, R. MORIKAWA, N. ISOGAI, Y. ASAKURA, Y. TORIDE and J. TAKAHASHI. 2004a. Effect of *Escherichia coli* W3110 or *Escherichia coli* nir-Ptac on *in vitro* ruminal methanogenesis and nitrate/nitrite reduction. *Proc. of 11th Asian-Australasian Association of Animal Production Societies (AAAP) Congress, 5 – 9th September 2004, Kuala Lumpur, Malaysia pp.* 316 – 318.
- SAR, C., B. SANTOSO, Y. GAMO, T. KOBAYASHI, S. SHIOZAKI, K. KIMURA, H. MIZUKOSHI, I. ARAI and J. TAKAHASHI. 2004b. Effects of combination of nitrate with β 1-4 galacto-oligosaccharides and yeast (*Candida kefyr*) on methane emission from sheep. *Asian-Aust. J. Anim. Sci.* 17: 73 – 79.
- SAR, C., B. SANTOSO, B. MWENYA, Y. GAMO, T. KOBAYASHI, R. MORIKAWA, K. KIMURA, H. MIZUKOSHI and J. TAKAHASHI. 2004c. Manipulation of rumen methanogenesis by the combination of nitrate with β 1-4 galacto-oligosaccharides or nisin in sheep. *Anim. Feed Sci. Technol.* 115: 129 – 142.
- SAR, C., B. MWENYA, B. PEN, K. TAKAURA, R. MORIKAWA, A. TSUJIMOTO, K. KUWAKI, N. ISOGAI, I. SHINZATO, Y. ASAKURA, Y. TORIDE and J. TAKAHASHI. 2005a. Effect of ruminal administration of *Escherichia coli* wild type or a genetically modified strain with enhanced high nitrite reductase activity on methane emission and nitrate toxicity in nitrate-infused sheep. *Br. J. Nutr.* 94: 691 – 697.
- SAR, C., B. MWENYA, B. SANTOSO, B. TAKAURA, K. MORIKAWA, R. ISOGAI, N. ASAKURA, Y. TORIDE and J. TAKAHASHI. 2005b. The effect of *Escherichia coli* W3110 on ruminal methanogenesis and nitrate/nitrite reduction *in vitro*. *Anim. Feed Sci. Technol.* 118: 295 – 306.
- SAR, C., B. MWENYA, B. SANTOSO, K. TAKAURA, R. MORIKAWA, N. ISOGAI, Y. ASAKURA, Y. TORIDE and J. TAKAHASHI. 2005c. Effect of *Escherichia coli* wild type or its derivative with high nitrite reductase activity on ruminal methanogenesis and nitrate/nitrite reduction *in vitro*. *J. Anim. Sci.* 83: 644 – 652.
- SAR, C., B. MWENYA, B. PEN, R. MORIKAWA, K. TAKAURA, T. KOBAYASHI and J. TAKAHASHI. 2006. Effect of nisin on ruminal methane production and nitrate/nitrite reduction *in vitro*. *Aust. J. Agric. Res.* 56: 803 – 810.
- SAR, C., B. SANTOSO, X.G. ZHOU, Y. GAMO, A. KOYAMA, T. KOBAYASHI, S. SHIOZAKI and J. TAKAHASHI. 2002. Effects of β 1-4 galacto-oligosaccharide (GOS) and *Candida kefyr* on nitrate-induced methaemoglobinemia and methane emission in sheep. *In: Greenhouse Gases on Animal Agriculture.* TAKAHASHI, J. and B.A. YOUNG (Eds). Elsevier, Amsterdam pp. 179 – 184.

- TAKAHASHI J., Y. GAMO, B. MWENYA, B. SANTOSO, C. SAR, H. UMETSU, H. MIZUKOSHI, K. KIMURA and O. HAMAMOTO. 2003. Control and energetic recycling of methane emitted from ruminants. Proc. of Progress in research on energy and protein metabolism. EAAP publication, No. 109, 13 – 18th September 2003, Rostock-Warnemünde, Germany pp. 371 – 374.
- TAKAHASHI, J. 2001. Nutritional manipulation of methanogenesis in ruminants. Asian-Aust. J. Anim. Sci. 14: 131 – 135.
- TAKAHASHI, J. 2006. Sustainable manure management in the nitrogen recycling system FLRC/NZFMRA. Conference on Implementing Sustainable Nutrient Management Strategies in Agriculture, Massey University, Palmerston North, New Zealand.
- TAKAHASHI, J. 2007. Feed production from unused resources applied ammonia stripping from digested slurry of biogas plant. J. Agric. Food Technol. 3: 3 – 5.
- TAKAHASHI, J. and B. A. YOUNG. 1991. Prophylactic effect of L-cysteine on nitrate-induced alteration in respiratory exchange and metabolic rate in sheep. Anim. Feed Sci. Technol. 35: 105 – 113.
- TAKAHASHI, J. and B. A. YOUNG. 1992. The modulation of nitrate-enhanced hypothermia by sulphur compounds in cold-exposed sheep. Anim. Feed Sci. Technol. 39: 347 – 355.
- TAKAHASHI, J. and M. UEMURA. 2009. Ammonia fuel cell modulated ammonia stripping from animal agricultural biomass. J. Fuel Cell Technol. 8: 93 – 96.
- TAKAHASHI, J., B. MWENYA, B. SANTOSO, C. SAR, K. UMETSU, T. KISHIMOTO, K. NISHIZAKI, K. KIMURA and O. HAMAMOTO. 2004. Mitigation of methane emission and energy recycling in animal agricultural system. Proc. of International Symposium on Recent Advances in Animal Nutrition, 7th September 2004, Kuala Lumpur, Malaysia pp. 97 – 103.
- TAKAHASHI, J., C. SAR, Y. GAMO, K. KIMURA, H. MIZUKOSHI and I. ARAI. 2002. Suppression of rumen methanogenesis by alternative reductions. Proc. of 10th International Congress, 23 – 27th September, 2002, New Delhi, India p. 55.
- TAKAHASHI, J., M. IKEDA, S. MATSUOKA and H. FUJITA. 1998. Prophylactic effect of L-cysteine to acute and subclinical nitrate toxicity in sheep. Anim. Feed Sci. Technol. 74: 273 – 280.
- TAKAHASHI, J., N. JOHCHI and H. FUJITA. 1989. Inhibitory effects of sulphur compounds, copper and tungsten on nitrate reduction by mixed rumen micro-organisms. Br. J. Nutr. 61: 742 – 748.
- TAKAHASHI, J., Y. MIYAGAWA, Y. KOJIMA and K. UMETSU. 2000. Effects of *Yucca schidigera* extract, probiotics, monensin and L-cysteine on rumen methanogenesis. Asian-Aust. J. Anim. Sci. 13: 499 – 501.
- UMETSU, K., Y. KIMURA, and J. TAKAHASHI. 2005. Methane emission from stored dairy manure slurry and slurry after digestion by methane digester. Anim. Sci. J. 76: 73 – 79.
- UMETSU, K., S. YASMAZAKI, and J. TAKAHASHI. 2006. Anaerobic co-digestion of dairy manure and sugar beets. In: Greenhouse Gases and Animal Agriculture: An Update. SOLIVA, R.C., J. TAKAHASHI and M. KREUZER (Eds.). Elsevier Sci. pp. 307 – 310.
- VAN NEVEL C. J. and D. I. DEMEYER. 1996. Control of rumen methanogenesis. Environ. Monit. Assess. 42:73 – 97.
- WALLES, R.J., L. ARTHAUD and C. J. NEWBOLD. 1994. Influence of *Yucca schidigera* extract in ruminal ammonia concentrations and ruminal microorganisms. Appl. Environ. Microbiol. 60: 1762 – 1767.
- WANG, Y., T.A. MCALLISTER, L.J. YANKE, Z. J. XU, CHEEKE P.R. and K.J. CHENG. 2000. *In vitro* effects of steroidal saponins from *Yucca schidigera* extract on rumen microbial protein synthesis and ruminal fermentation. J. Sci. Food Agric. 80: 2114 – 2122.
- WOLIN, M.J. and T.L. MILLER. 1988. Microbe-microbe interactions. In: The Rumen Microbial Ecosystem. HOBSON, P.J. (Ed.). Elsevier Appl. Sci., London, UK pp. 343 – 359.