

Ruminant Nutrition II: Methane

208 Dynamics of volatile fatty acids, hydrogen, and methane in dairy cattle: A model of rumen metabolic pathways. H. J. van Lingen^{*1,2}, J. G. Fadel³, L. E. Moraes⁴, E. Kebreab³, A. Ban-nink², and J. Dijkstra², ¹*Ti Food and Nutrition, Wageningen, the Netherlands*, ²*Wageningen University & Research, Wageningen, the Netherlands*, ³*University of California, Davis, Davis, CA*, ⁴*Ohio State University, Columbus, OH*.

Most rumen mechanistic models adopt a zero pool for hydrogen and estimate methane production based on hydrogen sources and sinks. A dynamic mechanistic model that represented substrate degradation, volatile fatty acid (VFA) production pathways, and methanogenesis in the bovine rumen was developed. This preliminary model also represented the thermodynamic control of H₂ partial pressure (p_{H_2}) on the type of VFA formed via the NAD⁺ to NADH ratio (r_{NAD}). Feed composition and intake rate (twice-daily feeding regimen) observations were used as model input. Model parameters were estimated to experimental data using a Bayesian calibration procedure, after which the uncertainty of the parameter distribution on the model output was assessed. This Bayesian mechanistic modeling effort is unique in providing a mathematical representation of diurnal dynamics of VFA, H₂ and CH₄ production in the bovine rumen, in which the type of VFA is controlled by p_{H_2} via r_{NAD} homeostasis, based on principles of reaction kinetics and thermodynamics. The preliminary model predicted a marked peak in p_{H_2} after feeding that rapidly declined in time. This peak in p_{H_2} caused a decrease in r_{NAD} followed by an increased propionate molar proportion at the expense of acetate molar proportion. In response to feeding, the model predicted an increase in CH₄ production that steadily decreased in time. The pattern of CH₄ emission rate followed the patterns of p_{H_2} and H₂ emission rate, but its magnitude of increase in response to feeding was less pronounced. A global sensitivity analysis was performed to determine the impact of parameters on daily CH₄ production. The parameter that determines the NADH oxidation rate explained 41% of the variation in predicted daily CH₄ emission. The preliminary model was evaluated using 40 measurements from 3 experiments conducted at Wageningen University. Model evaluation indicated daily CH₄ production to be under-predicted, and showed a root mean square prediction error of 15%. The present modeling effort provides the integration of more detailed knowledge than in previous rumen fermentation models and allows assessment of diurnal dynamics of rumen metabolic pathways yielding VFA, H₂ and CH₄.

Key Words: thermodynamic control, Bayesian calibration, mechanistic modeling

209 Effects of 3-nitrooxypropanol on rumen fermentation, lactational performance, and onset of ovarian activity in dairy cows. A. Melgar^{*1}, M. T. Harper¹, J. Oh¹, F. Giallongo¹, M. E. Fetter¹, T. L. Ott¹, S. Duval², and A. N. Hristov¹, ¹*The Pennsylvania State University, University Park, PA*, ²*Research Centre for Animal Nutrition and Health, DSM Nutritional Products, France*.

This study investigated the effect of 3-nitrooxypropanol (3NOP), a methane (CH₄) inhibitor, on rumen fermentation, lactational performance, and reproductive function in Holstein dairy cows. The study was a 15-wk randomized complete block design with 56 multi- and primiparous cows (including 8 rumen-cannulated). Cows were blocked based on their previous lactation milk yield or predicted milk yield and within block were randomly assigned to one of 2 treatments: (1) control, no 3NOP,

and (2) 3NOP applied at 60 mg/kg feed DM. Cows entered the study within 3 d after calving and remained on the study for 105 d. Enteric CH₄ emission was measured during experimental wks 2, 6, 9, and 15 using the GreenFeed system. Rumen samples for fermentation analyses were collected during wks 4, 8, and 12. Milk was sampled 3 d/wk from 2 to 8 wks after calving and assayed for progesterone concentration to determine onset of ovarian activity. Compared with the control, 3NOP decreased total VFA and acetate, increased butyrate molar proportion, ethanol, and formate ($P \leq 0.05$), and tended ($P = 0.14$) to increase dissolved hydrogen concentrations in the rumen. CH₄ emission was decreased 25% by 3NOP (337 vs. 225 g/d for the control; $P < 0.001$). 3NOP also decreased ($P < 0.001$) CH₄ emission yield (14.0 vs. 11.2 g/kg DMI) and intensity (8.3 vs. 6.3 g/kg ECM). DMI was lower for 3NOP compared with the control (23.7 vs. 24.9 kg/d; $P = 0.05$), but DMI as % of BW was not different (4.0%; $P = 0.94$) between treatments. Treatment had no effect ($P \geq 0.63$) on milk and ECM yields, averaging 44 kg and 41.7 kg/d, respectively. Feed and ECM feed efficiencies were increased ($P \leq 0.01$) by 3NOP compared with the control. Milk composition and milk fat and protein yields were not affected ($P \geq 0.39$) by treatment. There was no effect ($P \geq 0.14$) of 3NOP on return to ovarian activity, days to first and second CL, and length of the first and second luteal phases. In this experiment, 3NOP decreased enteric CH₄ emission, yield, and intensity without affecting milk yield and composition or onset of ovarian activity in early lactation dairy cows.

Key Words: 3-nitrooxypropanol, dairy cattle, reproduction

210 Dose-response effect of 3-nitrooxypropanol on enteric methane emission in dairy cows. A. Melgar^{*1}, K. C. Welter², K. Nedelkov³, C. M. M. R. Martins², M. T. Harper¹, J. Oh¹, S. E. Räisänen¹, X. Chen⁴, S. F. Cueva⁵, S. Duval⁶, and A. N. Hristov¹, ¹*The Pennsylvania State University, University Park, PA*, ²*University of Sao Paulo, Pirassununga, Brazil*, ³*Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria*, ⁴*College of Pastoral Agriculture Science and Technology, Lanzhou University, China*, ⁵*Panama American Agricultural University, Zamorano, Francisco Morazan, Honduras*, ⁶*Research Centre for Animal Nutrition and Health, DSM Nutritional Products, France*.

This experiment was designed to test the effect of inclusion rate of 3-nitrooxypropanol (3NOP), a methane inhibitor, on enteric methane emission in dairy cows. The study was conducted with 49 multiparous cows (155 ± 7.7 DIM, 634 ± 8.1 kg BW, and 44 ± 1.1 kg/d milk yield) in a randomized complete block design. The experiment was conducted in 2 phases; phase 1 was with 21 cows and phase 2, with 28 cows. Cows were blocked based on DIM, milk yield, and enteric methane emission during a 14-d covariate period. Cows were fed ad libitum a basal TMR diet containing (% DM basis): corn silage, 50; alfalfa haylage, 10; and concentrate feeds and mineral-vitamin premix, 40. Treatments were control (placebo, no 3NOP) and 40, 60, 80, 100, 150, and 200 mg 3NOP/kg feed DM. Following a 14-d adaptation period, enteric gaseous emissions (methane, carbon dioxide, and hydrogen) were measured in 3 d using the GreenFeed system (C-Lock Inc., Rapid City, SD) at 0900, 1500, and 2100 h (d 1), 0300, 1200, and 1700 h (d 2), and 0000 and 0500 h (d 3). Dry matter intake (24.6 kg/d; SEM = 1.12), milk yield (44.0 kg/d; SEM = 1.15), and cow BW (634 kg; SEM = 5.2) were not affected ($P \geq 0.33$) by treatment. 3NOP linearly decreased ($P < 0.001$) methane emission by an average of 31% (303 vs. 441 g/cow/d for the control; SEM = 25.7). The 3 highest 3NOP inclusion rates had a similar

methane mitigation effect (average of 267 g/d, or a 40% reduction; $P \geq 0.93$). 3NOP linearly decreased ($P < 0.001$) methane yield (average of 12.6 vs. 17.0 g/kg DMI for the control; SEM = 1.15) and methane emission intensity (7.1 vs. 9.7 g/kg milk, respectively; SEM = 0.63). Emission of hydrogen linearly increased ($P < 0.001$) from 0.3 g/cow/d for the control to an average of 2.2 g/cow/d (SEM = 0.42) for the 3 highest 3NOP inclusion rates. Emission of carbon dioxide was not affected by treatment (average of 13.7 kg/cow/d; SEM = 6.16; $P = 0.97$). In this experiment, 3NOP linearly decreased enteric methane production in dairy cows. Maximum mitigation effect was achieved at 100 mg/kg feed DM with no further decrease in enteric methane emission with 150 and 200 mg/kg 3NOP.

Key Words: 3-nitrooxypropanol, enteric methane, dairy cattle

211 Effect of limit-feeding diets with different forage to concentrate ratios on the fecal bacterial and archaeal community composition in Holstein heifers. J. Zhang*, H. Shi, Y. Wang, Z. Cao, and S. Li, *State Key Laboratory of Animal Nutrition, Beijing Engineering Technology Research Center of Raw Milk Quality and Safety Control, College of Animal Science and Technology, China Agricultural University, Beijing, China.*

Limit-fed high concentrate diet was proposed as an effective method to improve feed efficiency and decrease manure output in heifers' raising, while the effect on hindgut microbiota was still unclear. Therefore, this study aims to investigate the effects of wide range of dietary forage:concentrate ratios (F:C) on the compositions of fecal bacteria and archaea in heifers. Twenty-four Holstein dairy heifers with similar body condition (8–10 mo old) were assigned into 4 treatments and fed diets with different F:C (80:20, 60:40, 40:60, and 20:80, DM basis). The quantity of diets provided to high concentrate fed groups was restricted so that there was a similar intake of metabolizable energy to low concentrate fed groups. From d 20 to 23, fecal samples were collected from the rectum of heifers at 3-h intervals. At the end of sampling, equal amount of sample of each time point from the same heifer were homogenized, and the subsamples were used for nutrient and volatile fatty acids detection and DNA extraction. The fecal bacterial and archaeal communities were investigated by Illumina sequencing. With increasing dietary concentrate levels, the fecal neutral detergent fiber and acetate contents linearly decreased ($P < 0.01$), while fecal starch, propionate, and butyrate contents increased ($P \leq 0.05$). An increased concentrate level linearly increased ($P = 0.02$) *Proteobacteria* abundance, while linearly decreased ($P = 0.02$) *Bacteroidetes* abundance in feces. At the genus level, cellulolytic bacteria, namely unclassified *Ruminococcaceae* and *Paludibacter*, were linearly decreased ($q \leq 0.02$), while non-fibrous carbohydrate degraders, namely *Roseburia* and *Succinivibrio*, were linearly increased ($q \leq 0.05$). However, the abundance of most detected archaeal were similar across different F:C, and only *Methanospaera* were linearly decreased ($P = 0.01$) with increasing concentrate levels. These results suggest that different F:C diets have significant effects on the fecal bacterial communities, while have slight effect on the archaeal communities. Our study might further be beneficial to enhance the feed digestibility and relieve environment problems in heifers' rearing.

Key Words: bacteria, archaea, heifer

212 Effect of essential oil extracted from tropical and/or sub-tropical plants on in vitro dry matter digestibility, ruminal fermentation, and methane production. D. H. Kim*^{1,2}, I. M. Ogunade¹, K. G. Arriola¹, D. Vyas¹, and A. T. Adesogan¹, ¹*Department of Animal Sciences, Institute of Food and Agricultural Sciences,*

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The objective was to examine the effects of essential oils (EO) from *Syzygium aromaticum* (clove), *Cinnamomum zeylanicum nees* (cinnamon), *Rosmarinus officinalis* (rosemary), *Zingiber officinale* (ginger), and *Cymbopogon citratus* (lemon grass) on in vitro dry matter digestibility (IVDMD), ruminal fermentation and methane production using a TMR as substrate. A corn silage-based TMR was treated with each EO at doses of 0 (CON), 200 (Low), 400 (Med) and 600 (High) mg/L of buffered rumen fluid and with Monensin (MON) at 12 mg/kg substrate DM (positive control). A randomized complete block design with 3 replicates per treatment and 3 runs (blocks) was used to determine the effects of EO and MON. Data for each essential oil were separately analyzed with the GLIMMIX procedure of SAS and treatment effects were estimated using multiple comparisons. Each treated substrate was incubated in a 120-mL gas-tight culture bottle at 39°C for 24 h. The EO had no effect on IVDMD when compared with the CON. However, compared with MON, IVDMD was increased ($P < 0.05$) by the low dose of rosemary and ginger. Gas volume was reduced ($P < 0.05$) by the low dose of rosemary and low and high doses of cinnamon, compared with CON. All EOs except lemon grass reduced ($P < 0.05$) methane production at all doses when compared with the CON. The high dose of rosemary increased ($P < 0.05$) pH compared with CON and MON. Lactate content was significantly higher ($P < 0.05$) in med dose of cinnamon. Acetate concentration was reduced ($P < 0.05$) by medium and high doses of clove and cinnamon while isobutyrate concentration was increased with all doses of clove and ginger, compared with CON and MON. The high dose of lemon grass reduced ($P < 0.05$) propionate concentration compared with the MON. The high dose of cinnamon increased ($P < 0.05$) butyrate concentration compared with the CON and MON. In conclusion, compared with the CON, all doses of EOs reduced in vitro methane production without reducing digestibility whereas MON had no effect on digestibility, methane production or gas production.

Key Words: essential oil, methane, digestibility

214 Methane inhibition following fermentation and microbiota community response by different dose of chitosan in vitro. J. Tong*¹, H. Zhang¹, L. Jiang¹, and B. Xiong², ¹*Beijing Key Laboratory for Dairy Cow Nutrition, Beijing University of Agriculture, Beijing, China, ²State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Chitosan, a non-toxic and biodegradable carbohydrate polymer, had diverse potential applications in the food processing, pharmaceutical, cosmetics, veterinary, environmental protection, agricultural industries and medicine. However, there is limited knowledge with regard to the different dose of chitosan in ruminant nutrition and whether chitosan is related to the altered bacterial community is still unclear. A completely randomized design was applied in vitro incubation. A basal diet with no chitosan was the control, and the treatment diets were supplemented with chitosan at the dose of 0.4, 0.8, 1.2, 1.6, and 2.0% DM. Six fermentation units were used per treatments. The concentrations of methane and volatile fatty acids were determined using a gas chromatograph. To explore methane inhibition following fermentation and the response of ruminal microbiota to different dose of chitosan, the bacterial 16S rRNA genes were sequenced by Miseq high-throughput sequencing and were confirmed by realtime-PCR. The results revealed that the dose of 2.0% chitosan had greatest reduction in methane emission and shifted the fermentation pattern toward propionate production. Real-time PCR

analysis showed that chitosan increased the population of *Fibrobacter succinogenes* and *Megasphaera* spp. relative to total bacterial 16S rDNA, while the dose of 2.0% CHI significantly increased the population of *Ruminococcus albus* compared with control ($P < 0.01$). Furthermore, 0.4% CHI could significantly increase the population of *Succinivibro* spp. ($P < 0.01$), but 2.0% CHI had no effect. ANOSIM showed no significant differences in bacterial community composition among all the treatments ($R = -0.03$, $P = 0.71$). Chitosan addition had a tendency of increase the feed digestibility and significantly increased the VFA production ($P < 0.10$). In particular, correlation analyses between relative abundances of bacteria genera showed that the propionate concentration was positively related with *Prevotellaceae* and *Bacteroidales* ($P < 0.05$), but negatively correlated with *Ruminococcaceae* ($P < 0.05$). Therefore, the effects of chitosan on microbial populations and bacterial communities, particularly on propionate production, are probably responsible for the discrepancy in its effects on rumen fermentation and methane emission, and thus it may be used as a potential alternative to fed to ruminants.

Key Words: chitosan, methanogenic, rumen fermentation

215 Relationship between residual feed intake and CH₄ production in dairy heifers. H. Flay*^{1,2}, B. Kuhn-Sherlock¹, K. Macdonald¹, M. Camara¹, D. Donaghy², N. Lopez-Villalobos², and J. R. Roche^{1,3}, ¹DairyNZ, Hamilton, New Zealand, ²Massey University, Palmerston North, New Zealand, ³University of Auckland, Symonds St, Auckland, New Zealand.

There is growing interest in improving feed conversion efficiency, through reducing residual feed intake (RFI), and in reducing agricultural methane (CH₄) emissions. As CH₄ is a major source of digestible energy loss in ruminants, it is plausible that selection for low RFI (i.e., high feed efficient) would also reduce CH₄ emissions. CH₄ production (g/d) and yield (g/kg DMI) for 56 heifers (20–22 mo old) were measured in a 2 × 2 factorial arrangement, including 2 breeds of dairy cattle (Jersey: Jer and Holstein-Friesian: HF; n = 28/breed) previously designated as either high (+2.0 kg DM) or low RFI (–2.1 kg DM; n = 28/RFI category). Breed × RFI category interactions were not significant; main effects are presented (see Table 1). HF heifers were significantly heavier and consumed more than Jer, but breed did not significantly affect DMI/kg BW or daily BW gain (BWg). Because of the lower DMI, Jer heifers produced less CH₄/d, but not per kg DMI or per kg BWg. RFI category had no significant effect on BW or BWg, but DMI and DMI/kg BW were 9.2% and 10.6% less in low RFI heifers. RFI category had no significant effect on CH₄/d or CH₄/kg BWg; but CH₄/kg DMI was greater in low RFI heifers because of their lower DMI. Results probably reflect more complete digestion of ingested feed in low RFI heifers, consistent with previously reported greater physical breakdown of feed and a higher

apparent digestibility of organic matter in low RFI animals. In conclusion, selecting dairy heifers for low RFI is unlikely to affect daily CH₄ production (g/d), but could increase CH₄ yield (g/kg DMI). *This research was funded by the New Zealand Government to support the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases.*

Key Words: feed conversion, environmental sustainability, greenhouse gas

216 Variation in animal performance explained by the rumen microbiome or by diet composition. C. B. Gleason* and R. R. White, Virginia Tech, Blacksburg, VA.

Although the rumen microbiome is thought to impact feed digestion, the connection between microbiome and production in beef and dairy cattle remains unclear. The aim of this meta-analysis was to determine if the microbiome can serve as an accurate predictor of animal performance compared with predictions based on diet composition. To support this comparison, a set of models was derived and compared. Models predicted milk yield (MY), ADG, DMI, and feed efficiency (FE) using different sets of independent variables: diet (D), microbiome (M), and experimental methods (EM). Diet independent variables included dietary percentages of dry and organic matter, neutral and acid detergent fiber, crude protein, ether extract, non-fiber carbohydrate, starch, and forage. Microbiome variables included relative abundance of 3 major bacterial phyla, species richness, and species diversity. Experimental variables were publication year, breed type (dairy, beef, or *Bos indicus*), and rumen sampling fraction (fluid or solid). A second set of models used D and EM variables as predictors of the microbiome. Predictor variable sets were used individually and in combination. Linear mixed-effects regression, weighted by standard error, was used to derive models using data from 51 journal articles. Models were compared on the basis of CCC, root estimated variance associated with study and error, and AICc, where appropriate. The MY model using D+M+EM predictors outperformed all other MY models (CCC = 0.71). Average daily gain was most accurately predicted by D alone (CCC = 0.92). Interestingly, M+EM was more successful at predicting DMI than any model using D. Similarly, dairy FE was more accurately predicted by M+EM than D, albeit slightly (CCC = 0.69 vs. 0.65), while beef FE could only be modeled using D variables. Breed type proved a better predictor of phyla than D. Conversely, species richness and diversity indicators were unaffected by breed type, but could be predicted by D. This analysis concludes that, in some cases, the microbiome may serve as an accurate indicator of animal performance independent of diet.

Key Words: rumen microbiome, diet, production

Table 1 (Abstr. 215). LSM (± SEM) for BW, DMI, BWg, and CH₄ in Jer and HF heifers from low or high RFI categories

| | BW, kg | DMI, kg/d | BWg, kg/d | CH ₄ , g/d | CH ₄ , g/kg DMI | CH ₄ , g/kg BWg |
|----------|-----------|-------------|------------|-----------------------|----------------------------|----------------------------|
| Jer | 408 ± 7.0 | 11.3 ± 0.29 | 1.2 ± 0.06 | 242 ± 4.3 | 21.6 ± 0.51 | 219 ± 9.6 |
| HF | 479 ± 7.0 | 12.4 ± 0.29 | 1.3 ± 0.06 | 267 ± 4.3 | 21.9 ± 0.51 | 211 ± 9.6 |
| <i>P</i> | <0.001 | 0.01 | 0.09 | <0.001 | 0.65 | 0.57 |
| Low RFI | 448 ± 9.8 | 11.3 ± 0.29 | 1.2 ± 0.06 | 253 ± 4.9 | 22.7 ± 0.47 | 222 ± 9.5 |
| High RFI | 439 ± 9.8 | 12.4 ± 0.29 | 1.3 ± 0.06 | 256 ± 4.9 | 20.7 ± 0.47 | 208 ± 9.5 |
| <i>P</i> | 0.50 | <0.01 | 0.31 | 0.63 | <0.01 | 0.30 |

217 Relationships between mean rumen pH and time spent under pH 5.8. D. M. Liebe*¹, J. L. Firkins², and R. R. White¹, ¹*Virginia Tech, Blacksburg, VA*, ²*The Ohio State University, Columbus, OH*.

Efforts focused on developing recommendations for how to prevent subacute ruminal acidosis (SARA) have identified nonlinearities between time spent below a threshold pH and mean ruminal pH in terms of their interpretation of likelihood of SARA. The goals of this study were to 1) define a relationship between mean rumen pH and time spent below pH 5.8 (TU5.8) and 2) to investigate how diet and cow characterizations influence TU5.8 when formulating a ration for a mean rumen pH between 6.0 and 6.1. Exponential models were fit with mean rumen pH as an independent variable and TU5.8 as the dependent variable. Metadata on rumen conditions were collected from 16 previously published studies (60 treatment means). Nonlinear least-squares regression was used to derive parameters for an exponential curve fitted through the center of the treatment means. Parameters of this curve were used as informative priors for a Bayesian hierarchical model. A Markov Chain Monte Carlo (MCMC) method was then used to sample randomly from each prior distribution and develop posterior estimates for the 2 parameters of our exponential equation: Φ_1 and Φ_2 within each study. Using these study-specific parameters derived by the MCMC approach as independent variables, linear models were fit to predict Φ_1 and Φ_2 using accompanying physical and chemical dietary data from each study. The rate of increasing TU5.8 was 30% greater at pH 6.0 than pH 6.1. The influence of dietary characteristics was determined by changing each parameter ± 1 SD and measuring area under the curve between 6.0 and 6.1 (AUC) compared with the mean diet. The most influential diet characteristics were DIM, CP, and butyrate concentration, changing AUC for TU5.8 by -6.1% , $+3.5\%$, and $+2.1\%$ at $+1$ SD, respectively. Whereas increasing DIM appears to reduce TU5.8 between mean pH 6.0 and 6.1, increasing dietary CP and ruminal butyrate concentrations appear to increase this TU5.8 the most of the variables studied, although other variables were significant. Irrespective of diet, the targeting of mean rumen pH of 6.0 to 6.1, through dietary changes such as forage chop-size, is recommended to avoid SARA.

Key Words: subacute ruminal acidosis, rumen pH, fiber

218 Preparing and characterizing magnetic nanoparticles coated with cellulose for effective enrichment of cellulolytic microorganisms from rumen. L. Xing^{1,2}, S. G. Zhao*^{1,2}, N. Zheng^{1,2}, and J. Q. Wang^{1,2}, ¹*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural*

Sciences, Beijing, China, ²*Key Laboratory of Quality & Safety Control for Dairy Products of Ministry of Agriculture, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China*.

Cellulolytic bacteria are considered to be one of the most important parts in rumen. However, it is still lack of effective approaches to enrich or isolate those uncultured cellulolytic bacteria. The magnetic nanoparticle-mediated isolation is a new method to isolate or enrich the uncultured bacteria. This research aimed to isolate and enrich ruminal cellulolytic bacteria. The magnetic nanocomposites (MNP) were synthesized and coated with cellulose. Transmission electron microscopy, X-ray diffraction, and value stream mapping were applied to characterize the characterization of MNP. MNP coated with cellulose (MNPC) (17.4 mg/mL) was mixed with *S. bovis* (1.0×10^8 cfu/mL) for 20 min to evaluate its capture efficiency, and for 60 h to evaluate the bacteria stability on MNPC. MNPC was mixed with *Cellulomonas flavigena* (*C. flavigena*, cellulolytic bacterium) and *Streptococcus bovis* (*S. bovis*, non-cellulolytic bacterium) for 60 h incubation to evaluate the isolation specificity of this approach. The bacterial in liquid were sucked out every 12 h and were determined by qPCR. To apply the method to enrich rumen cellulolytic bacteria, MNPC was mixed with rumen bacteria and incubated for 90 h. The bacteria in liquid were sucked out every 10 h and were determined by qPCR. Results showed MNP were globular and had average size of 20 nm and magnetization of 3.3–24.9 emu/g. The capture efficiency of MNPC was 99%. The stability of *S. bovis* on the MNPC was 90.24% after 60 h. The enriched *C. flavigena* raised from 6.49×10^2 cfu/mL to 2.20×10^6 cfu/mL while *S. bovis* could not metabolize cellulose and captured by the MNPC with 1.05×10^3 cfu/mL in the liquid. Relative abundance of the typed rumen cellulolytic bacteria, e.g., unclassified *Butyrivibrio*, unclassified *Fibrobacter*, and *R. flavefaciens* increased from 1.06%, 0.01%, 0.10% to 7.02%, 0.09%, 0.26%, respectively after 90 h incubation. The growth trend of a species from uncultured *Lachnospiraceae* (relative abundance of 28.33%) is related to that of the typed cellulolytic bacteria, so it was indicated that this species was a potential cellulolytic bacterium. This study provided a new approach to cultivate the uncultivable cellulolytic microorganisms.

Key Words: enrichment, magnetic nanoparticles