



CENTRAL LUZON STATE UNIVERSITY



**ACETOGENIC AND ACID UTILIZING BACTERIAL CONTENT ANALYSIS
ON FORESTOMACH FLUID OF WATER BUFFALO
(*Bubalus bubalis*) CALVES**

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An Undergraduate Thesis Submitted to the Faculty of the Department of
Biological Sciences, College of Arts and Sciences Central Luzon
State University, Science City of Muñoz, Nueva Ecija,
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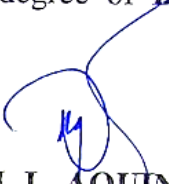
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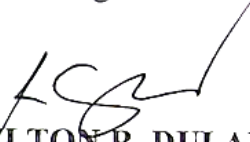
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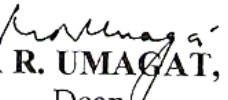

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ABSTRACT

ROCHE ZHES G. ROBENIOL, Bachelor of Science in Biology, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, June 2017. **ACETOGENIC AND ACID UTILIZING BACTERIAL CONTENT ANALYSIS ON FORESTOMACH FLUID OF WATER BUFFALO (*Bubalus bubalis*) CALVES**

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Microbial digesta of water buffaloes was investigated to isolate, characterize and differentiate the morphological and cultural analysis of acetogenic and acid utilizing bacteria present from the digestive tract of the calves. This is done through feeding with associated different diets upon the calves. The alteration of the feeds diet (raw milk and supplemented feeds diet) from the birth of the calves until the 30th was been observed in the interval of four samplings (day1, 6th, 16th, 16th and 30th). Samples was isolated anaerobically through anaerobic chamber in avoidance with exposure to environment then incubated at 37⁰C for 24-48 hours for visible growth of microorganisms.

The identified genera of bacteria are *Oscillospira sp.*, *Ruminococcus sp.* and *Acetobacterium sp.* as the acetogens and acid utilizers are *Sarcina sp.*, *Salmonella sp.*, *Streptococcus sp.*, *Desulfovibrio sp.*, *Micrococcus sp.*, and *Streptobacillus sp.* The bacterial population is consistently increasing as the calves grow older. There is no



significant difference between the two diets fed to the weaning calf which concludes that replacement of diet is not affected in the weaning of calves.

In proximate analysis of feeds, it has total fiber of 8.50%, total protein of 22.53%, total fat of 7.09% and ash with 6.41% while the component of Pakchong variety of grass fed to the weaning calves has an approximate percentage of 58%-61% of hemicelluloses (NDF), has 7%-11% crude protein and 8%-12% ash. Calves ability to digest feeds began as their rumen is modified throughout their weaning which leads to the development of their monogastric stomach into a modified rumen. However, starter feeds and grass contributes to the quality of the rumen that stimulates the environment and invigorates the ability of the rumen to conform growth and digest more.



LITERATURE CITED

- ABDL-RAHMAN, M.A. (2010).** *In vitro* Manipulation of Rumen Fermentation Efficiency by Fumaric acid – Bentonite Coupled Addition as an Alternative to Antibiotics. *Journal of Agricultural Science*. 2(2): 174-180.
- AHMED, K. and D.K. BHATTACHARYA. (2005).** Isolation, Identification and Antibioqram of Aerobic Bacteria from Repeat Breeding Buffaloes. *Indian Veterinary Journal*, 82:898-899.
- ARYAL, S. (2003).** Biochemical Test of *Streptococcus pyogenes*. *Microbiology Information*. 13-15.
- ALUWONG, T., WUYEP, P. and L. ALLAM. (2011).** Livestock-environment interactions: Methane emissions from ruminants African *Journal of Biotechnology*. *Academic Journals*, 10(8):1265-1269.
- AMLAN, K., PATRA, E. and S. JYOTISNA. (2009).** Dietary phytochemicals as rumen modifiers: a review of the effects on microbial populations. *Springer Science Business Media*. 96:363–375.
- BAS, P., ARCHIME`DE, H., ROUZEAU, A. and D. SAUVANT. (2003).** Fatty Acid Composition of Mixed-Rumen Bacteria: Effect of Concentration and Type of Forage. *Journal of Dairy Science*, 86:2940–2948.
- BAUMAN, D.E., HARVATINE, K.J. and A.L. LOCK. (2011).** Nutri-genomics, rumen- derived bioactive fatty acids, and the regulation of milk fat synthesis. *Annual Revolution Nutrition*, 31:299–319.
- BRYANT, M.P., WOLIN, E.A., WOLIN, M.J. and R.S. WOLFIE. (1967).** *Methanobacillus omelianskii*, a Symbiotic Association of Two Species of Bacteria. *Archive for Microbiology*. 59, 20-31.
- CHANTHAKHOUN, V., WANAPAT, M. and S. WACHIRAPAKORN. (2011).** Effect of legume (*Phaseolus calcaratus*) hay supplementation on rumen microorganisms, fermentation and nutrient digestibility in swamp buffalo. *Livestock Science*, 140:17–23.
- CLARKE, R.T.J. (1979).** Niche in Pasture-Fed Ruminants for the Large Rumen Bacteria *Oscillospira lampropedi*. *Applied and Environmental Microbiology*. 37(3): 657-667.



- COOK, A.R. (1976).** Urease Activity in the Rumen of Sheep and the Isolation of Ureolytic Bacteria. *Journal of General Microbiology* (1976), 9, 32-48.
- COSTELLO, R. (2012).** Bloat in Young Calves and Other Pre-ruminant Livestock. Merrick's Inc.
- DEHORTY, B.A. and C.G. ORPIN.(1997).** Development of and natural fluctuations in, rumen microbial populations. *The Rumen Microbial Ecosystem*. Hobson PN and Stewarts, 196-245.
- DENEV, S. A., PEEVA,T.Z., RADULOVA, P., STANCHEVA, P., STAYKOVA, G., BEEV, G., TODOROVA, P. and S. TCHOBANOVA. (2007).** Yeast Cultures in Ruminant Nutrition *Journal of Agricultural Science*. 13: 357-374.
- EDWARDS, R. and R. G. FINCH. (1986).** The Pathological Society of Great Britain and Ireland Characterisation and antibiotic susceptibilities of *Streptobacillus moniliformis*. *Journal of Medical Microbiology*. Vol. 21.
- FERNANDO, S.C. (2008).** Meta-Functional Genomics of the Bovine Rumen. *Animal Genetics* 1:345.
- FONTY, G., GOUET, P. and J.M. NEBOUT. (1989).** Development of the Cellulolytic Microflora in the Rumen of Lambs Transferred into Sterile Isolators a Few Days After Birth. *Journal of Microbiology*, 35:416 – 422.
- FORTINA, F.O., TASSONE, S. BODAS, R. and LOPEZ, S. (2012).** Effects of inactivated and live cells of *Saccharomyces cerevisiae* on in vitro ruminal fermentation of diets with different forage: concentrate ratio. *Journal of Agricultural Science*. 150, 271–283.
- FORSBERG, C. W. (1990).** Sulfide production from cysteine by *Desulfovibrio desulfuricans*. *Applied and Environmental Microbiology*. Vol. 39:2.
- FREII, C., WIEDERHOLD,N. and D. BURGESS.(2008).**Antimicrobial Breakpoints for Gram-Negative Aerobic Bacteria Based On Pharmacokinetic Pharmacodynamic Models With Monte Carlo Simulation *Journal of Antimicrobial Chemotherapy*, 61: 621–628.
- GREGG, K., ALLEN, G. and C.BEARD. (1996).** Genetic Manipulation of Rumen Bacteria: from Potential to Reality. *Australian Journal of Agricultural Research*, 47:247-56.



- GOLLOP, N. ZAKIN, V. and Z.G. WEINBERG. (2006).** Antibacterial activity of lactic acid bacteria included in inoculants for silage and in silages treated with these inoculants. *Journal of Applied Microbiology*, 8, 662–666.
- GUAN, L.L., NKRUMAH, J.D., BASARAB, J.A. and S.S. MOORE. (2008).** Linkage of microbial ecology to phenotype: correlation of rumen microbial ecology to cattle's feed efficiency. *Microbiology Literature*, 288:85–91.
- GYLSWYK, V. and E.G. ROCHE (1970).** Characteristics of Ruminococcus and Cellulolytic Butyrivibrio Species from the Rumens of Sheep Fed Differently Supplemented Teff (*Eragrostis tef*) Hay Diets. *Journal of General Microbiology*, 64: 11-17
- HAL, J.K., LEE, S.S., GOTO, M., MOON, Y.H. and K.J. CHENG. (2002).** Influence of Tween 80 on the Enzyme Distribution in Rumen Liquor and on the Growth of Rumen Bacteria and Fungi. *Journal of Applied Animal Research*, 21: 129-143.
- HUNGATE, R. E. (1966).** The Rumen and its microbes. Department of Bacteriology and Agricultural Experiment Station. University of California. Davis. Academic Press, 1-533.
- HARIHARANA, H., MATTHEWA, V., FOUNTAINA, J., SNELLA, A., DOHERTYA, D., KINGA, B., SHEMERA, E., OLIVEIRAB, O. and R. SHARMAA. (2011).** Aerobic bacteria from mucous membranes, ear canals, and skinwounds of feral cats in Grenada, and the antimicrobial drug susceptibility of major isolates. *Comparative Immunology, Microbiology and Infectious Diseases*, 34:129–134.
- HEINRICHS, J. (2005).** Rumen Development in the Dairy Calf. *Advances in Dairy*, 17: 179.
- HUDSON, J.A, CAL, Y. MORVAN, C.B. and JOBLIN, K.J. (2000).** Identification and enumeration of oleic acid and linoleic acid hydrating bacteria in the rumen of sheep and cows. *Journal of Applied Microbiology*, 88, 286–292.
- IMAI, S., ABDULLAH, N., HO, Y.W., JALALUDIN,S., HUSSAIM, H.Y., ONODERA, R. and H. KUDO. (2000).** Comparative Study on the Rumen Ciliate Populations in Small Experimental Herds of Water Buffalo and Kedah Lekantah Cattle in Malaysia. *Animal Feed Science and Technology*, 52: 345-353.
- INGALE, S.L., LOKHAND, E, A. and S. ZADBUKE. (2013).** Nutritional strategies to mitigate greenhouse gases emission from livestock agriculture: a review. *Livestock Research International*. 1:2;34-45.



- ITAL, J., REDDY, P.G., MORRILL, J.L. and H.C. MINOCHA. (1985).** Effect of early weaning on the cell mediated immune response of dairy calves. Nutritional Reproduction. International 31:501. Animal Science, 6:2, 50-507.
- JIMENEZ, J.R. (1996).** Isolation of endospore-forming bacteria from marine sources. Journal of Marine endospore-forming bacteria. 1-16.
- KALAIVANI, R. and V. SUKUMARAN. (2013).** Characterization of Bacterial Diversity in Marine Sediment at Different Seasons in Karankadu, Tamilnadu, Indian International Journal of Pure Applied Zoology, 1(1): 97-108.
- KALLE, G.P. and K. K. G. MENON. (1984).** Inhibition of methanogenesis and its Reversal during biogas formation from cattle manure. Journal of Biological Science, 6:315-324.
- KATARIA, R.P. (2015).** Use of feed additives for reducing greenhouse gas emissions from dairy farms. Microbiology Research, 6:6120.
- KHANAL, R.C. and T.R. DHIMAN. (2004).** Biosynthesis of Conjugated Linoleic Acid (CLA): A Review. Pakistan Journal of Nutrition, 3(2): 72-81.
- KHEJORSART, P., WANAPAT, M. and P. ROWLINSON. (2011).** Diversity of anaerobic fungi and rumen fermentation characteristic in swamp buffalo and beef cattle fed on different diets. Livestock Science, 139:230-236.
- KOBAYASHI, Y., WAKITA, M. and S. HOSHINO. (1993).** Intracellular Amino Acid Profile of Rumen Bacteria as Influenced by Urea Feeding and its Duration. AJAS, 4:619-622.
- KOCUR, M. and T. MARTINEC. (1959).** The Taxonomic Study of *Sarcina Aurantiaca* Fluegge. International Bulletin of Bacteriological Nomenclature and Taxonomy. 9(4): 165-168.
- KOIKE, S. and Y. KOBAYASI. (2001).** Development and use of competitive PCR assays for the rumen cellulolytic bacteria : *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*. FEMS Microbiology. Literature. 204 : 361-366.
- KONGMUN, P., WANAPAT, M., PAKDEE, P., NAVANUKRAW, C. and Z. YUB. (2011).** Manipulation of rumen fermentation and ecology of swamp buffalo by coconut oil and garlic powder supplementation. Livestock Science, 135: 84-92.



- KUDO, H., CHENG,K., ABDULLAH, N., HUSSAIN, H., HO, Y. and S. JALALUDIN. (1994).** Effects of Chemical Treatments of Barley Straw on Leaching and Digestibility by Rumen and Cellulytic Bacteria. *Applied Journal of Animal Science*. 3:389-3896.
- LAMBE, D.W., ALEXANDER, M. MCPHEDRAN, MERTZ, J. A. and S. PHYLLIS. (1973).** *Streptobacillus moniliformis* Isolated from a Case of Haverhill Fever: Biochemical Characterization and Inhibitory Effect of Sodium Polyanethol Sulfonate. *Applied Journal Chemistry and Physics*; Vol. 60.
- LEE, S.Y., YANG, H.S., LEE, S.W., KIM, S., SHIN, D.E and J.K. HA. (2009).** Effect of 2 Bromoethanesulfonic Acid on *In vitro* Fermentation Characteristics and Methanogen Population. *Asian-Australian Journal of Animal Science*. Vol. 22, No. 1: 42 – 48.
- LEEDLE, J.A., BRYANT, M.P. and R.B.HESPELL. (1952).** Diurnal Variations in Bacterial Numbers and Fluid Parameters in Ruminal Contents of Animals Fed Low- or High-Forage Diets. *Applied and Environmental Microbiology*, (44); 2:402-412.
- LEEDLE, J.A., BRYANT, M.P. and R. HESPELL. (1980).** Diurnal variations in Bacterial numbers and fluid parameters in ruminal contents of animals fed low- or high forage diets. Division of Microbiology, Department of Dairy Science, University of Illinois.
- LESMEISTER, K.E., HEINRICHS, A.J. and M.T. GABLER. (2004).** Effects of Supplemental yeast (*Saccharomyces cerevisiae*) culture on rumen development, growth characteristics, and blood parameters in neonatal dairy calves. *Journal of Dairy Science*, 87:1832-1839.
- LESMEISTER, K.E., TOZER, A.J. and A.J. HEINRICHS.(2004).** Development and Analysis of a Rumen Tissue Sampling Procedure. *Journal of Dairy Science*. 87:1336-1344.
- LI, R.W., CONNOR, E.E., LI, C., BALDWIN, V.I. and M.E.SPARKS.(2012).** Characterization of the rumen microbiota of pre-ruminant calves using meta-genomic tools. *Environmental Microbiology*. 14:129 –139.
- LONGENBACH, J.I. and A.J. HEINRICHS. (1998).** A review of the importance and physiological role of curd formation in the abomasum of young calves. *Animal Feed Science and Technology*,73:8597.



- MACKIE, R.I., AMINOV, R.I., WHITE, B.A. and C.S. MCSWEENEY.(2000).** Molecular ecology and Diversity in gut microbial ecosystems. Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction.
- MANIMEGALAI, R., GOPINATH, L.R., CHRISTY, MERLIN and D. DIVYA (2003).** Isolation and Identification of Acetogenic and Methanogenic Bacteria from Anoxic Black Sediments and their Role in Biogas Production. International Journal of Plant, Animal and Environmental Sciences. 53;1609-1614.
- MCEVOY, J.M., DOHERTY, A.M. SHERIDAN, J.J., BLAIR, I.S. and D.A. MCDOWELL. (2003).** The prevalence of Salmonella spp. in bovine faecal, rumen and carcass samples at a commercial abattoir. Journal of Applied Microbiology. 94, 693-700.
- MACKIE, R.I. (1997).** Gut Environment and Evolution of Mutualistic Fermentative Digestion. Gastrointestinal Microbiology, 13-35.
- MARTIN, S., BALCELLS, J., ZAKARAOUI, F. and C. CASTRILLO. (1997).** Quantification and Chemical Composition of Mixed Bacteria Harvested from Solid Fractions of Rumen Digesta: Effect of Detachment Procedure. Animal Feed Science and Technology, 71: 269-282.
- MARTIN, C., DEVILLARD, E.D. and M. DOREAU.(1999).** Influence of Sampling Site on Concentrations and Carbohydrate-Degrading Enzyme Activities of Protozoa and Bacteria in the Rumen. Journal of Animal Science, 77:979-987.
- MCCANN, J., WICKERSHAM, T. and J. LOOR. (2014).** High-throughput Methods Redefine the Rumen Microbiome and Its Relationship with Nutrition and Metabolism Bioinformatics and Biology Insights, 8:109-125.
- MIRZAEI, A. and N.M. SIS. (2016).** Factors affecting mitigation of methane Emission from ruminants: Microbiology and biotechnology strategies. Animal Behavior of Biological Meteorology, 4(1): 22-31.
- MINA, B.R., ATTWOOD, T., MCNABBB, W.C., MONLAB, A.L. and T.N. BARRY. (2005).** The effect of condensed tannins from *Lotu scorniculatus* on the proteolytic activities and growth of rumen bacteria. Animal Feed Science and Technology, 121:45-58.
- MORRILL, J.L. (1984).** Research in dairy calf nutrition procedure. Cornell Nutrition Conference, 46-50.



- PRASAD, A.L.N. and LITWACK, G. (1965).** Growth and Biochemical Characteristics of *Micrococcus lysodeikticus*, Sensitive or Resistant to Lysozyme. *Biochemistry* 4(3):496-500.
- REDDY, P.G., MORRILL, J.L. and H.C. MINOCHA. (1985).** Effect of early weaning on the cell mediated Immune response of dairy calves. *Nutrition*. 31:501.
- REY, M., ENJALBERT, F., COMBES, S., CAUQUIL, L., BOUCHEZ, O. and V. MONTEILS. (2014).** Establishment of ruminal bacterial community in dairy calves from birth to weaning is sequential. *Journal of Applied Microbiology*, 116:245–257.
- ROMEO, J. (1979).** A Dichotomous Key for the Identification of Miscellaneous Gram-Negative Bacteria. *Laboratory Medicine*. 10(9). 548-558.
- RUSSELL, J.B., O'CONNOR, J.D., FOX, D.G., VAN, P.J., SOEST, P.J. and C.J. SNIFFEN. (1992).** A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *Journal of Animal Science*, 70(11):3551–61.
- RUSSELL, J.B., MUCK, R.E. and P.J. WEINER. (2009).** Quantitative analysis of cellulose degradation and growth of cellulolytic bacteria in the rumen. *Microbiological Ecology*, 67 (2009) 183–197.
- SAMPLES, O.M. (2010).** Rumens Fluid Analysis. Kentucky Veterinary Medical Association, mid- America Veterinary Conference, 19(10):35-41.
- SANDHU, H. and M. BAL. (1997).** Toxicological Effects of Triazophos on Rumens Micro-organisms in Buffalo Calves. *Indian Veterinary Journal*, 74: 648-651.
- SHAMBULINGAPPA, B. E. and G.A.MANEGAR. (2010).** Aerobic Bacterial Flora and Antibioqram Profile in Canine Metritis. *Indian Veterinary Journal*, 556 – 558.
- SHARDA, R. and R. KRISHNAN. (2001).** Aerobic Bacterial Flora of Cervical Mucus of Repeat Breeding Cattle and Buffaloes. *Indian Veterinary Journal*, 78:659-650.
- SINGH, N. (1972).** Studies on the effect of two different management practices on the establishment of rumen microbial activity in the buffalo calves. *Agra University. Journal of Research Science*, 21:2:85.
- SURV, G.B. (2003).** Identification of Salmonella. *Laboratory Protocols*. 4th Edition.
- SOTO, R., SAMIRAH, A., MUHAMMED, C., NEWBOLD, C.S., STEWART, R. and K. WALLACE. (1994).** Influence of Peptides, amino acids and urea on



microbial activity in the Rumen of Sheep Receiving Grass Hay and on the Growth of Rumen Bacteria in Vito. *Animal Feed Science Technology*, 49: 151-161.

STEWART, C.S., FLINT, H.J. and M.P. BRYANT. (1997). The rumen bacteria. *The Rumen Microbial Ecosystem*, 10–72.

SUNGA, H.G., BAEA, H.D., LEEA, J.H., KIMB, D.K. and T.H. SHINA.(2004).Application of LDH enzyme and viable LDH-producing bacteria to prevent lactate accumulation during in vitro rumen fermentation. *Animal Feed Science and Technology*, 117:235–243.

SURYANINGSIH, N.A., RAHIM, S.A., JAMIL, H. and A.ARFAN. (2015). Isolation and Identification of *Desulfovibrio sp.* Bacteria from Acid Sulfate Soil. *Asian Journal of Applied Sciences* (2321 – 0893).

TAJIMA, K., AMINOROV, R.I., NAGAMINE, T., MATSUI, H., NAKAMURA, M. and Y. BENNO. (2001). Diet dependent shifts in the bacterial population of the rumen revealed with real time PCR. *Applied Environmental Microbiology*, 67: 2766-2774.

TAMATE, H., MCGILLIARD, A.D., JACOBSON, N.L. and R. GETTY. (1962). Effects of various dietaries on the anatomical development of the stomach in the calf. *Journal of Dairy Sci.*, 45:408- 420.

TAN, H.Y., SIEO, C.C., ABDULLAH, N., LIANG, J.B., HUANG, X.D. and Y.W. HO. (2011). Effects of condensed tannins from *Leucaena* on methane production, rumen fermentation and populations of methanogens and protozoa in vitro. *Animal Feed Science Technology*, 169(3–4): 185–93.

TERRE, M., CASTELLS, L., FABREGAS,F. and A. BACH. (2013). Short communication: comparison of pH, volatile fatty acids, and microbiome of rumen samples from preweaned calves obtained via cannula or stomach tube. *Journal of Dairy Science*, 96:5290 –5294.

TERRAMOCCIA, S., BARTOCCI, S., AMICI, A. and F. MARTILLOTTI. (2000). Protein-free dry matter rumen degradability in Cattle and Sheep fed-diets with different forage to concentrate ratios. *Livestock Production Sciences*, 65: 185-185.

THOETKIATTIKUL, H., MHUANTONG, W., LAOTHANACHAREON, T., TANGPHATSORNRUANG, S., PATTARAJINDA, V., EURWILAICHITR, L. and V. CHAMPREDA. (2013). Comparative analysis of microbial profiles in cow rumen fed with different dietary fiber by tagged 16S rRNA gene pyrosequencing. *Current Microbiology*, 67(2):130-7.



- VAN, N.O. and ROCHE, E.G. (1970).** Characteristics of *Ruminococcus* and Cellulolytic *Butyrivibrio* Species from the Rumen of Sheep Fed Differently Supplemented Teff (*Eragrostis tef*) Hay Diets. *Journal of General Microbiology*, 64, 11-17.
- VAREL, V.H., YEN, J.T. and K.K. KREIKMEIER. (1995).** Addition of Cellulolytic Clostridia to the Bovine Rumen and Pig Intestinal Tract. *Applied and Environmental Microbiology*, (61):3; 1116-1119.
- VENTOSA, A., NIETO, J.J. and A. OREN. (1998).** Biology of Moderately Halophilic Aerobic Bacteria. *Microbiology and Molecular Biology Reviews*. *Journal of Dairy Science*, (62); 2:504-544.
- VIJAYARANI, K., NACHIMUTHU, K. and V.D. PADMANABAN.(2000).** Isolation of Cellulolytic Bacteria from Bovine Rumen. *Indian Veterinary Journal*, 77:662-654.
- WANAPAT, M., NGARMSANG, A., KORKHUNTOT, N., NONTASO, C. WACHIRAPAKORN, D. and G. P. ROWLINSON.(1999).** A comparative study on the Rumen Microbial Population of Cattle and Swamp Buffalo Raised under Traditional Village Conditions in the Northeast of Thailand. *Department of Microbiology, Faculty of Science*, 56:62-64.
- WANAPAT, M., PILAJUN, R. and S. KONGMUN. (2006).** Ruminant ecology of swamp buffalo as influenced by dietary sources. *Animal Feed Science and Technology*, 151:205-214.
- WANAPAT, M., WORA-ANU, S., WACHIRAPAKORN, C. and N. NONTASO. (2007).** Effect of Roughage Sources on Cellulolytic Bacteria and Rumen Ecology of Beef Cattle. *Asian-Australian Journal of Animal Science*, (20); 11:1705-17112.
- WANAPAT, M., and P. ROWLINSON. (2007).** Nutrition and feeding of swamp buffalo: feed resources and rumen approach. *Italian Journal of Animal Science*, vol. 6, (2), 67-73.
- WANG, X., LI, X., ZHAO, C., HU,P.,CHEN,H.,LIU,Z., LIU, G. and Z.WANG. (2012).** The correlation between composition of the bacterial community and concentration of volatile fatty acids in the rumen during the transition period and ketosis in dairy cows. *Applied Environmental Microbiology*,78:2386-2392.
- WARREN, R. M., CITRON, D., MERRIAM, V.C., and J.C. GOLDSTEIN. (2005).** Biochemical Differentiation and Comparison of *Desulfovibrio* Species and Other Phenotypically Similar Genera. *Journal of Clinical Microbiology*. 43(8): 4041-4045.



YANG, C.L., GUAN, L.L., LIU, J.X. and WANG J.K. (2015). Rumen fermentation and acetogen population changes in response to an exogenous acetogen TWA4 strain and *Saccharomyces cerevisiae* fermentation product. *Journal of Zhejiang University-Science (Biomedicine & Biotechnology)*. 1862-1783.

YANAGITA, K., MANOME, A. MENG, X.Y., HANADA, S., KANAGAWA, T. TSUCHIDA, T., MACKIE, R. and Y, KAMAGATA. (2003). Flow cytometric sorting, phylogenetic analysis and in situ detection of *Oscillospira guillermondii*, a large, morphologically conspicuous but uncultured ruminal bacterium. *International Journal of Systematic and Evolutionary Microbiology*. 53, 1609–1614.