



CENTRAL LUZON STATE UNIVERSITY



**ASSESSMENT OF CILIATE PROTOZOAN COMMUNITY ON THE DIGESTIVE
TRACT FLUID OF DOMESTIC WATER BUFFALO (*Bubalus bubalis*) CALVES
FED ON RAW MILK AND FORMULATED FEEDS DIET**

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An Undergraduate Thesis Submitted to the Faculty of the Department of
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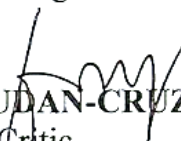
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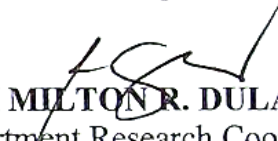
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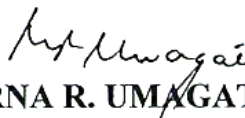

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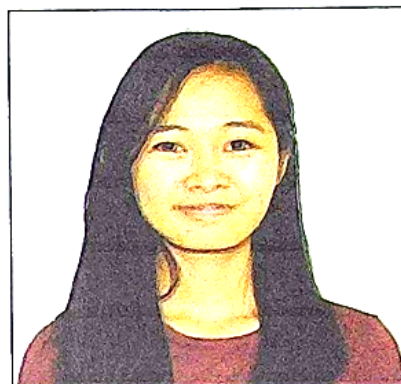

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ABSTRACT

CHRISTINE JOY PEREJA VEREÑA, 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. **ASSESSMENT OF CILIATE PROTOZOAN COMMUNITY ON THE DIGESTIVE TRACT FLUID OF DOMESTIC WATER BUFFALO (*Bubalus bubalis*) CALVES FED ON RAW MILK AND FORMULATED FEEDS DIET**

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This study aimed to isolate and identify morphologically the ciliate protozoa present on the calves' digestive tract fluid and determine the effect of milk and formulated feeds diet on the ciliate population on the digestive tract fluid of domestic buffalo calves'. Methylene-formaldehyde method of centrifugation was used to identify and determine the protozoa. There were eleven (11) morphologically identified species of ciliate protozoa namely *Entodinium minimum*, *Entodinium bifidum*, *Entodinium parvum*, *Entodinium nanellum*, *Entodinium biconcavum*, *Entodinium simplex*, *Charonia ventricoli* , *Blepharocorythia sp.*, *Dastricha ruminantum*, *Isotrichidae prostoma*, *Buetschilia sp.* , belonging to 4 genera namely Ophryoscolecidae, Blepharocorythidae, Buetschliidae, Isotrichidae and classified into 2 different groups of Holotrich and Entodiniomorphid protozoa. The ciliate protozoan population growth was higher on calves given alternative weaning diet all throughout the 30 days of observation.



LITERATURE CITED

- ANDERSON, K.L.T., NAGARAJA, G. and J.L. MORILL. (1978). Ruminant metabolic development in calves weaned conventionally or early. *Journal Dairy Science*. 70: 1000-1005.
- BAILEY, R. W., & HOWARD, B. H. (1963). Carbohydrases of the rumen ciliate *Epidinium caudatum* (Crawley). 2. α -Galactosidase and isomaltase. *Biochemical Journal*, 87(1), 146.
- BORHAMI, B.E., el -SHAZLY, A.K., ABOU AKKADA, A.R. and I.A. AHMED. (1967). Effect of early establishment of ciliate protozoa in the rumen on microbial activity and growth of early weaned buffalo calves, *Dairy Science*, 50: 10.
- CHAUDHARY, L.C. and A. SIRVASTAVA. (1994). Performance of growing murah buffalo calve as affected with treatment of manoxol and the presence of ciliate protozoa in the rumen. *Animal Feed Science and Tecnology*, 51: 281-296.
- CHOUDHURY, P. K., SALEM, A.Z.M., JENA, R., KUMAR, S., SINGH, R. and A.K. PUNIYA. (2015). Rumen microbiology: An overview. In *Rumen Microbiology: From Evolution to Revolution* (pp. 3-16). Springer India.
- CHURCH, D.C. (1998). *The ruminant animal. Digestive physiology and nutrition*, Pretice hall, Inc. Englewood Cliffs, New Jersey.
- CLARKE, J. (1963). The cultivation of some rumen oligotrich protozoa. *Microbiology*, 33(3), 401-408.
- COLEMAN, G.S. (1978). Rumen Entodiniomorphid Protozoa: Methods of cultivating Parasites in vitro. 39-54 Academic Press: London.
- COLEMAN, G.S. (1980). Cultivation of rumen entodiniomorphid protozoa on tropical animal feeds, *Tropical Animal Production*, 198: 6:1.
- COLEMAN, G.S., LAURIE, J.I., BAILEY, J.E. and S.A. HOLDGATE. (1976). The Cultivation of Cellulolytic Protozoa Isolated from the Rumen, *Journal of General Microbiology*, 95: 144-150.
- DEHORITY, B.A. (1993). *Laboratory manual for classification and morphology of rumen ciliate protozoa*. CRC Press.



- DEHORTY, B.A. and C.G. ORPIN. (1997).** Development of, and natural fluctuations in, rumen microbial populations. In *The rumen microbial ecosystem* (pp. 196-245). Springer Netherlands.
- DEHORITY, B. A. (1986).** Protozoa of the digestive tract of herbivorous mammals. *International Journal of Tropical Insect Science*, 7(03), 279-296.
- DPPFAOUN. (2015).** Dairy production and products, food and agriculture. Organization of the United Nations. Website: http://www.fao.org/agriculture/dairy-gateway/milk-production/en/#.VkT-_fmrTIU.
- FAKRUDDIN, M.D., CHOWDHURY, A., HOSSAIN, M.N. and R.M. MAZUMDAR. (2012).** *Pryosequencing-principles and applications*, Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh, ISSN 2250-0480.
- FEEDIPEDIA WEBSITE. (2016).** Animal Feed Resources Information System – International Nutrition Research Association on animal feeds and Food Association Organization©2012-2016. <http://www.feedipedia.org/node/395>, <http://www.feedipedia.org/node/8329>
- FOOD AND AGRICULTURAL ORGANIZATION WEBSITE. (2011).** <http://www.fao.org/docrep/003/x6552e/x6552e06.htm>
- FRANZOLIN, R. and B.A. DEHORITY. (2010).** The role of pH on the survival of rumen protozoa in steers. *Revista Brasileira de Zootecnia*, 39(10), 2262-2267.
- HARRISON, H.N., WARNER, R.G., SANDLER, E.G. and J.K. LOOSLI. (1960).** Changes in the tissue and volume of the stomach of calves following the removal of dry feed or consumption of inert block. *Journal Dairy Science*. 43:1301-1312.
- HEINRICHS, J. (2005).** Rumen development in dairy calf. In: *advances in dairy technology*, 17: 179-187.
- HUNGATE, R.E. (1966).** *The rumen and its microbes*. Department of Bacteriology and Agricultural Experiment Station. University of California. Davis, 1-533, Academic Press, New York.
- 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. *Nutrition reports international*.



- JARVIS, B. D. W. and R. E. HUNGATE. (1968). Factors influencing agnotobiotic culture of the rumen ciliate *Entodinium simplex* Applied Microbiology, 16: 044-1052.
- JOUANY, J.P. (1998). The role of protozoa in feed digestion-Review. Asian-Australasian Journal of Animal Sciences, 12(1), 113-128.
- KAMRA, D. N. (2005). Rumen microbial ecosystem, Microbiology Section, Centre of Advanced Studies in Animal Nutrition, Indian Veterinary Research Institute, Izatnagar 243 122, India, 89, 1, 10 2005.
- KOIKE, S. and Y. KOBAYASI. (2001). Development and use of competitive PCR assays for the rumen cellulytic bacteria: *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*. 204: 361-366.
- KOFOIDC, A. and F. MACLENNANR. (1932). Ciliates from Bosindicus Linn. 2. A revision of Diplodinium Schuberg. University of California Publications in Zoology, 37, 53-1 52.
- KONRAD, L., CAMPERO, L.M., CASPE, G.S., BRIHUEGA, B., DRAGHI, G., MOORE, D.P., CRUDELI, G.A., VENTURINI, M.C. and C.M. CAMPERO. (2003). Detection of antibodies against *Brucella abortus*, *Leptospira spp.*, and *Apicomplexa* protozoa in water buffaloes in the Northeast of Argentina, Tropical Animal Health Production (2013) 45:1751–1756
- KUMAR, A. (2010). Rumen Protozoa in different species of livestock, Indian Veterinary. Journal; 87:1166 – 1167.
- KUMAR, S., NAGARAJAN, M., SANDHU, J. S., KUMAR, N., BEHL, V. and G. NISHANTH. (2007). "Mitochondrial DNA analyses of Indian water buffalo support a distinct genetic origin of river and swamp buffalo" (PDF). Animal Genetics, 38: 227: 232.
- LEEDLE, J. A. Z., BRAYANT, M.P. and R.B. HESPEL. (1982). Diurnal Variations in Bacterial Numbers and Fluid Parameters in Ruminal Contents of Animals Fed Low- or High-Forage Diets, 44, 2.
- 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 Dairy Sci. 87:1832-1839.



- LESMEISTER, K.E., TOZER, P.R. and A.J. HEINRICHS. (2004).** Development and analysis of a rumen tissue sampling procedure. *Journal Dairy Science*. 87:1336-1344.
- LIGGENSTOFFER, A.S., YOUSSEF, N.H., COUGER, M.B. and M.S. ELSHASHED. (2010).** Phylogenetic diversity and community structure of anaerobic gut fungi (phylum Neocallimastigomycota) in ruminant and non-ruminant herbivores, *The ISME Journal*, 4, 1225–1235.
- 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. *Anim. Feed Science and Technology* 73:85-97.
- MACKIE, R.I. (1997).** Gut environment and evolution of mutualistic fermentative digestion. *Gastrointestinal Microbiology*, I 13-35. Chapman and Hall, New York.
- MORRILL, J.L. (1984).** Research in dairy calf nutrition. In *Cornell Nutrition Conference. Feed Manufacture*. Syracuse, New York (p. 46).
- NOCEK, J.E., HEALD, C.W. and C.E. POLAN. (1984).** Influence of ration physical form and nitrogen availability on ruminal morphology of growing bull calves. *Journal dairy Sci*. 67:334-343.
- NYRÉN, P. (2001).** Method for sequencing DNA based on the detection of the release of pyrophosphate and enzymatic nucleotide degradation. Patents: 6: 258: 568 and 98/28440.
- OLUMEYAN, D.B., NAGARAJA, T.G., MILLER, G.W., FREY, R.A. and J.E. BAYER. (1986).** Rumen Microbial Changes in Cattle Fed Diets with or without Salinomycin, *Journal of Applied Microbiology*, 51: 2.
- PHOTO CITATION FOR CALF DIGESTIVE TRACT DIAGRAM WEBSITE:**
<http://thesouthdakotacowgirl.com/check-the-ingredients-of-your-calf-milk-replacer-now/>. To book: 605.222.5088
- RAMÍREZ, J.F., MEDINA, S. and N. GARCÍA. (2007).** Effects of the supplementation with yeast (*saccharomyces cerevisiae*) on weight gain and development of water buffalo calves. Center for Research on Sustainable Farming Systems, Cipav. Carrera 25 6-62 Cali 1806-2636.
- 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 Reproduction International*. 31:501.



REGENSBOGENOVA, M., KISIDAYOVA, S., MICHALOWSKI, T., JAVORSKY, P., MOON-VAN DER STAAY, S. Y. M., MOON-VAN DER STAAY, G. W., HACKSTEIN, J. H. P., Mc EWAN, N. R., JOUANY, J.P., NEWBOLD, J. C. and P. PRISTAS. (2004). Rapid Identification of Rumen Protozoa by Restriction Analysis of Amplified 18S rRNA Gene, *ActaProtozool.* 43:219 – 224.

REVIEWS GLOSSARY FOR PYROSEQUENCING WEBSITE.
(2011).http://www.nature.com/nrg/journal/v6/n11/glossary/nrg1709_glossary

RHAMAN, S.A., PURSER, D.B. and W.J. TYZNIK. (1964). Cultivation of rumen protozoa 1 Factors influencing the cultivation of rumen oligotrich protozoa in vitro *Journal of Protozoology*, 11:51-SS.

ROSSI, M. F., MARTINELE, I. and M. D'AGOSTO. (2013). Quantitative and Differential analysis of ciliate protozoa in rumen content samples filtered before and after fixation, *Zootec.*, 42 : 11 : 831-834, 2013.

SALAMA, M.A. (1986). Physiological studies on digestive performance in buffalo calves. PhD Thesis. Fac. Agric. Zagazig, University, (Banha branch) Egypt.

SHIN, E.C., CHO, K.M., LIM, W.J., HONG, S.Y., AN, C.L., KIM, E.J., KIM, Y.K., CHOI, B.R., AN, J.M., KANG, J.M., KIM, H. and H.D. YUN. (2004). Phylogenetic analysis Of protozoa in the rumen contents of cow based on the 18S rDNA sequences, *Journal of Applied Microbiology*, 97, 378–383.

SINGH, S., BHATIA, S.K., SANGWAN, D.C. and A.D.VIDYASAGAR. (1992). *Technological bull. Indian. Journal Animal Science.*62: 1197.

SINGH, S., SANGWAN, D.C., SINGH, S. and K. PRADHAN. (1997). National Symposium on Feeding Strategy. For Eco- Friendly Animal. Production. In India, Febuary 14-15, 1997, Izatnagar.

SINGH, C. V. and R. S. BARWAL. (2010). Buffalo Breeding Research and Improvement Strategies in India. Pages 1024–1031 in the Buffalo in the World.Proceedings of the 9th World Buffalo Congress, Buenos Aires.

SINGH, K. M., TRIPATHI, A. K., PANDYA, P. R., RANK, D. N., KOTHARI, R. K. and C. G. JOSHI. (2011). Dasytricha Dominance in Surti Buffalo Rumen Revealed by 18S rRNA Sequences and Real-Time PCR Assay, *Current Microbiology* 63:281–288 DOI 10.1007/s00284-011-9975-4.

TAJIMA, K., AMINOROV, R.I., NAGAMINE, T., MATSUI, H., NAKAMURA, M. and Y. BENNO. (2011). Diet dependent shifts in the bacterial population of the rumen revealed with real time PCR. *Application Environmental Microbiology*, 67: 2766-2774.



- TAMATE, H., MCGILLIARDAD, N.L., JACOBSON, R. and R. GETTY. (1962).** Effects of various dietaries on the anatomical development of the stomach in the calf. *Journal dairy Science* 45:408- 420.
- VASQUES-ANON, M.A., HEIRICHS, J., ALDRICH, J.M. and G.A. B. VARGA. (1993).** Post- weaning age effects on rumen fermentation end products and Digesta kinetics in calves weaned at 5 weeks of age. *Journal Dairy Science* 76:2742-27-48. webmaster@cattletoday.com.
- VEIRA, D.M. (1986).** The Role of Ciliate Protozoa in Nutrition of the Ruminant, *Journal Animal Science*, 63:1547-1560.
- WALLACE, R. J. (2014).** Ruminant Microbiology, Biotechnology, and Ruminant Nutrition: Progress and Problems, *Journal Animal Science*. 72:2992-3003.
- WANAPAT, M., NGARMSANG, A., KORKHUNTOT, S., NONTASO, N., WACHIRAPAKORN, C., BEAKES, G. and P. ROWLINSON. (2000).** A comparative study on the rumen microbial population of cattle and swamp buffalo raised under traditional village conditioning the northeast of Thailand. *Asian-Australasian Journal of Animal Sciences*, 13(7): 918–921.
- WANAPAT, M. (2001).** Swamp buffalo rumen ecosystem and its manipulation. Proceeding buffalo workshop. Microbiology section, 18025, 332a.
- WARD, G A. (2008).** Effect of pre-weaning diets on lamb's rumen development. *American-Eurarian Journal Agriculture & Environmental Science*. 3 (4):561-567.
- WILLIAMS A. G. (1986).** Rumen Holotrich Ciliate Protozoa. Hannah Research Institute, Ayr KA6 5HL, Scotland. *Microbiological Reviews*, Mar. 1986, p. 25-49 Vol. 50, No. 1, 0146-0749/86/010025-25\$02.00/0, Copyright ©D 1986, American Society for Microbiology
- ZETINA-CORDOBA, P., ORTEGA-CERRILLA, M. E. , ORTEGA-JIMENEZ, E. ; HERRERA-HARO, J. G. , SANCHEZ TORRES-ESQUEDA, M. T. , RETA-MENDIOLA, J. L. , VILABOA-ARRONIZ, J. and MUNGUÍA-AMECA, G., (2013).** Effect of cutting interval of Taiwan grass (*Pennisetum purpureum*) and partial substitution with duckweed (*Lemna* sp. and *Spirodela* sp.) on intake, digestibility and ruminal fermentation of Pelibuey lambs. *Livestock. Science*. 157 (2–3): 471-477
- ZIOLECKI, A. and C.A.E. BRIGGS. (1961).** Themicroflora of the rumen of the young calf: II. Source, Nature and Development. *Journal Application Bacteriology*. 24:148.