

R4D *Highlights*



PCC showcases research efforts under new paradigm in 2015 R4D in-house review

By MA. CECILIA C. IRANG

The Philippine Carabao Center (PCC) is moving into a non-traditional, problem-oriented and focused Research for Development (R4D) that has more relevance to stakeholders in the carabao industry.

In the context of this new paradigm, PCC showcased its research efforts in this year's R4D in-house review held at the PCC national headquarters in the Science City of Muñoz, Nueva Ecija on June 16-17.

Focusing on the thematic areas on biosafety, genetic improvement-animal genomics, product development, production management system, genetic improvement-reproductive and cryopreservation

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techniques, and social and policy researches relevant to the carabao industry, the two-day activity presented five completed researches, 17 on-going studies and two special presentations.

The research papers were first presented in the R4D pre-in-house review conducted by PCC in February to screen on-going and completed researches prior to presentation in the in-house review.

The in-house review is an annual activity that demonstrates and recognizes the PCC's research initiatives. It likewise helps create opportunities for researchers and scientists to present their noteworthy accomplishments, and to interact and share learnings with one another.

Further, the in-house review is a monitoring and evaluation tool for PCC to ensure that its efforts on research for development are aligned with its R4D Agenda, according to Dr. Eric Palacpac, national R4D coordinator.

"It serves as a venue for sharing research results with the scientific community and as a motivating tool for researchers to strengthen their interest in conducting pertinent researches in line with the PCC's mandate," he stated.

The event was capped with a recognition program for the best paper and best presenter as its way of recognizing researchers who excel in their respective disciplines.

Dr. Jezie A. Acorda, professor at the College of Veterinary Medicine at the University of the Philippines – Los Baños (CVM-UPLB) chaired the panel of external evaluators that included Dr. Rosalina Lapitan, Scientist I and a retired PCC employee, and Peter James Icalia, instructor at the Mariano Marcos State University (MMSU).

"There are things that can be addressed by research with practical application that will contribute directly to solving our problems. Our continuing efforts, extension services and other activities are all focused on increasing farmers' income. How will the farmers benefit from all these? That is the bottom line; our R4D will help us lead to that increasing income. PCC, however, is consistent in delivering relevant researches on different field of disciplines and consistent also in delivering its targets to the government," Dr. Arnel N. Del Barrio, PCC acting executive director, said in his remarks during the review.

"We want to bundle all researches addressing industry problems like, for example, researches related to increasing milk yield among buffaloes, reducing



National R4D Coordinator Dr. Eric P. Palacpac discusses to the participants the research for development agenda of the Philippine Carabao Center.

mortality rate, increasing fertility, calf drop and the like," he added.

At the end of the two-day review, awards were given to the researchers who excelled in their research activities.

Adjudged best paper in the completed research category was the study titled, "The Kinetics of Sperm Penetration and Embryo Development as Predictors of Fertility of Frozen Buffalo Semen" conducted by Excel Rio Maylem, PCC science research specialist II.

The student thesis titled, "Biological Control Efficacy of the Nematophagous Fungi *Duddingtonia flagrans* in Common Strongyle Roundworms and *Fasciola sp.* of Swamp Buffaloes" conducted by Toni Rose M. Barroga with Dr. Claro N. Mingala, Scientist II as host researcher was given the

best paper and best presenter awards.

The special presentations were on two studies titled "Raw milk physico-chemical characteristics of moringa leaf meal (MoLM) fed to crossbred water buffaloes" conducted by Dr. Norberto Tadeo, a professor at the Isabela State University, and "Correlation of infrared tympanic and rectal body temperatures in lactating Bulgarian murrah buffaloes in the Philippines", conducted by Dr. Adrian Ybañez, dean of the School of Health and Sciences and associate professor at the Cebu-based Southwestern University (SWU).

Some 80 participants joined the in-house review. These included scientists and researchers from the PCC national and regional centers as well as students and research faculty from the state universities.

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Assessing semen quality: more objective, accurate thru computer-assisted sperm analysis

By MA. CECILIA C. IRANG

Reproduction starts with a good quality oocyte (egg cells) and a good quality semen.

This, as a general rule, is what researchers are steadfastly upholding in so far as the reproductive system is concerned. But they are almost always saddled with the problem of having to go through meticulous evaluation of sperm motility and quality to ensure utmost success.

This problem, though, can now be overcome auspiciously - thru the use of Computer-Assisted Sperm Analysis or CASA.

The CASA evaluates sperm motility objectively. It provides more accurate data on the speed motion characteristics than the conventional method of sperm motility evaluation which is a subjective microscopic approach.

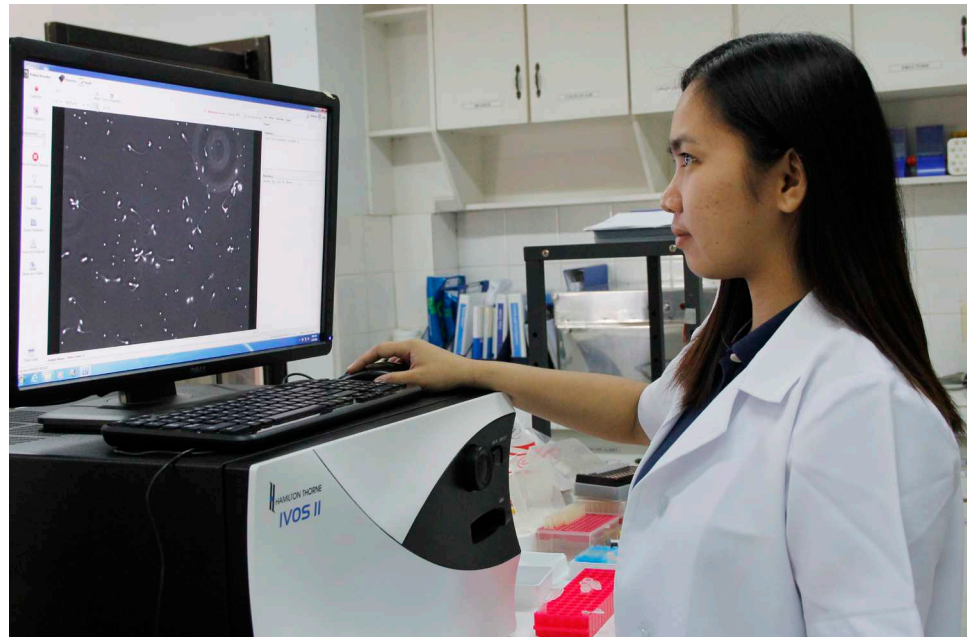
According to Ms. Excel Rio S. Maylem, lead researcher of the study titled, "Characterization of Sperm Kinematics by Computer-Assisted Sperm Analysis and Its Implication in Genetic Improvement of Water Buffaloes", conventional method of evaluating or assessing semen quality is just based on subjective observation. As it is widely carried out, it uses morphological description, qualitative approximation, and subjective motility grading which vary among technical staff and from laboratory to laboratory.

Advantage of CASA

The rise of CASA brought a new dimension to semen evaluation, Maylem said.

It provides a quantitative view of the number of moving sperms over the total number of all the sperms counted. It is known to be the most accurate, repeatable, and highly reliable method in determining the kinematics of ejaculates based on measurements of individual sperm cells in almost all species, experts said.

"We need innovation because CASA is now being used across the world to check the quality of the semen. CASA is more accurate as it is capable of determining the movement of a single sperm unlike the subjective method in which the sperm is just like going with the flow wherein



Ms. Excel Rio S. Maylem while using the Computer Assisted Sperm Analyzer (CASA).

you see the whole movement but not the individual movement of the sperm," Maylem explained.

It also gives a numeric value which is more reliable than the observation method, she added. In going about her study, Maylem collected, analyzed and cryopreserved semen samples from six buffalo bulls. Fresh and frozen semen were then analyzed with the CASA sperm kinematic parameters such as average path velocity, curvilinear velocity, straight line velocity, amplitude of lateral head displacement, beat cross frequency, straightness, linearity and wobble.

According to the research, CASA can provide three levels of classification. The first level was based on motility population, how many were motile, and are progressively motile, which cannot be determined when using subjective quality assessment. The second level, is based on the speed of movement, i.e., whether rapid, medium or slow. The third level, on the other hand, is the use of kinematic parameters that makes CASA really different from subjective method. "This is the main essence or value of CASA that others don't have. It can measure the sperm's speed from one point to another, the movement of its head, how wide and often they move and how straight they go," Maylem said.

In doing artificial insemination (AI), the

sperm's movement should be straight to reach the egg. In the subjective method, Maylem emphasized that they cannot determine the path of the moving sperm whereas thru the use of CASA they can see if the sperm is moving backward, straight or diagonal.

"The movement of the sperms present in the straw at post-thaw is of great value so as to ensure that it can reach the oocyte and be able to fertilize it following sperm deposition into the female reproductive tract," she said.

"When we use CASA, we can only select those with good quality sperms and maximize its use. Our future plan is to trace those bulls with good combination of the sperm movements for it to fertilize the egg," Maylem said.

Aside from that, the CASA can work across all species, like swine, goat, chicken, horse and even humans. It also has morphology feature that can detect normal and abnormal shapes of sperms.

The study was conducted to enable objective characterization of sperm motility and selection of outstanding buffalo semen donors for AI and eliminate undesirable ones through the use of CASA. In addition, the CASA system was used to enable the generation of different sperm populations

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TIM-3, GAL-9, NRAMP1 and NRAMP2: possible candidate genes responsible for disease resistance, tolerance, and/or susceptibility in WATER BUFFALOES

By MA. CECILIA C. IRANG

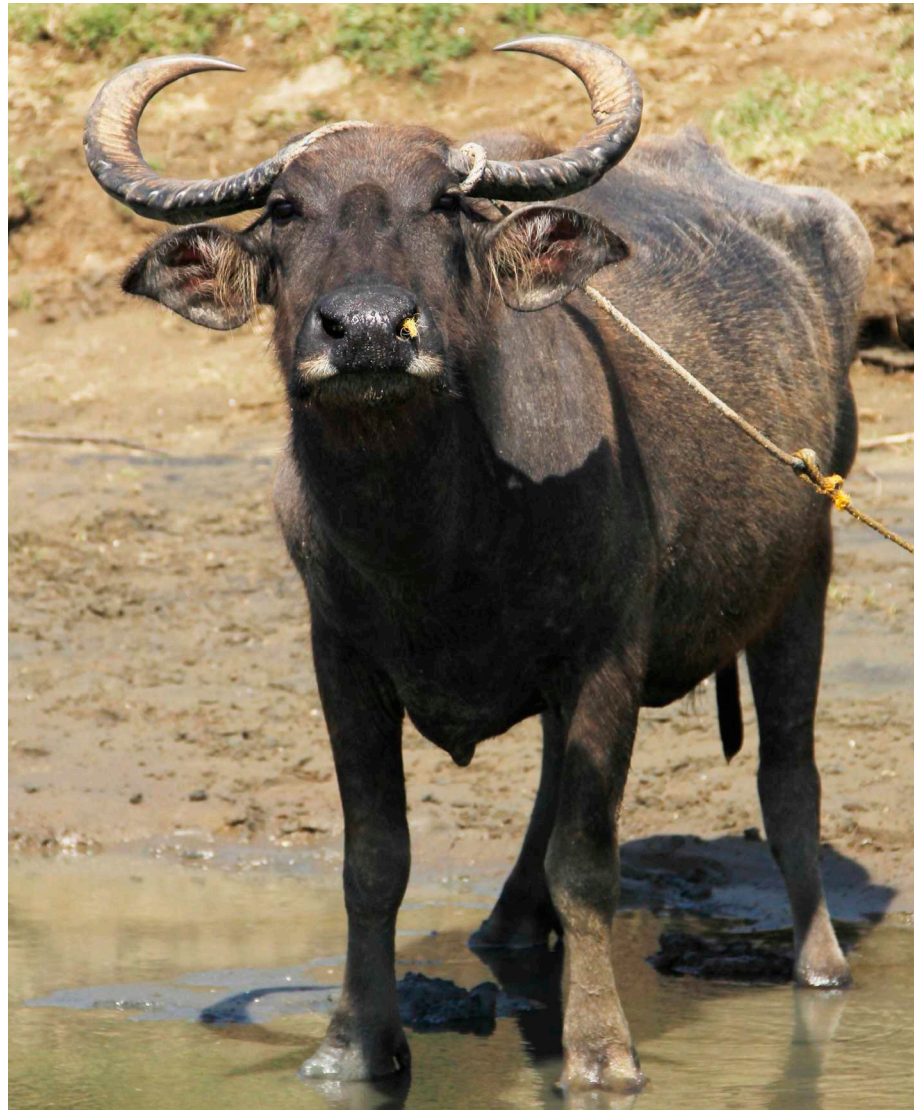
There are claims by several sectors that swamp-type water buffaloes are more resistant to infectious disease compared to riverine water buffaloes. To confirm this, the Philippine Carabao Center (PCC), through its Animal Health Unit, has conducted a preliminary study on the “Molecular Characterization of T-cell immunoglobulin mucin domain-3 (TIM-3), Galectin-9 (GAL-9), and NRAMP1/2 (Slc11a1/a2) genes of these two types of water buffaloes”.

Initial results of study indicated that there are unique changes in the amino acids of TIM-3, GAL-9, and NRAMP1/2 genes that can be associated with disease resistance tolerance and/or susceptibility in water buffaloes.

Swamp and riverine buffaloes are the two types of water buffalo. Swamp-type water buffaloes are the native water buffaloes or carabaos that are found in the Philippines and in the South of Southeast Asian nations while the riverine buffaloes are mainly found in India, Europe, and the Americas.

The importance of water buffalo to the lives of Filipino farmers is emphasized in the study as it is becoming a major source of milk that helps farmers increase their income and improve their over-all well-being. However, chronic occurrence of diseases is a major constraint in a dynamic and progressive livestock production and even in the meat-and-milk consuming human population.

Nevertheless, thru the introduction of genomic technologies and methods, the necessary tools are now available to begin identifying the genes that contribute to disease resistance, tolerance and/or susceptibility of the animal. The genes identified by these genomic studies provide valuable insight into disease biology and represent the initial steps



toward the efficient therapeutic strategies that can substantially improve the animal's health.

Characterization of genes

According to the study, TIM-3 is a type-1 glycoprotein which is persistently expressed on dysfunctional T-cells during chronic infection. It is typically found on dendritic cells (DC), natural killer cells, CD4+Th1 cells, monocytes, and cytotoxic T-lymphocytes (CTL). It is characterized as an immune response negative regulator. Meanwhile, GAL-9 is a versatile immune-modulator involved in a wide

range of biologic activities, such as cell adhesion and migration, proliferation and apoptosis, interaction of host cells with microbial pathogens, regulatory T-cell (Treg) differentiation and function, DC maturation, and antimicrobial immunity.

Whereas, solute-linked carrier 11a and 11a2 (Slc), also referred as Natural Resistance Associated Macrophage Protein 1/2, (Nramp1/Nramp2), have been associated to disease resistance and/or susceptibility across animal species. These genes have an important mechanism in the regulation against intracellular infection.

“Most of the farmers and even technical experts claim that native carabaos are disease-resistant or tolerant. This serves as one of our basis in conducting our study,” Ryan Bismark Padiernos, lead researcher of the study, said.

He added that previous studies proved that other animals are disease-resistant but as for buffaloes, none so far had been done.

“We want to know the immunological basis of determining disease-resistant, tolerant and/or susceptible buffalo. The first step we did was to describe the genetic characteristic of four genes (TIM-3, GAL-9, and NRAMP-1/2) in the immune system of the swamp and riverine water buffaloes,” he said.

The study also compared these four genes with other mammalian species and determined the unique characteristic specific in water buffalo to provide baseline information prior to the assessment of disease progression in buffalo species.

Methodologies employed

The researchers collected samples from Philippine Carabao (swamp type) and Bulgarian Murrah Buffalo (river type) to study the four genes.

Technically, materials and methods used in the study of these genes from swamp water buffaloes and riverine water buffaloes were Peripheral Blood Mononuclear Cell (PBMC) Isolation and Cultivation, Extraction of RNA and RT-PCR, Cloning, Sequencing and Homology and Phylogenetic analysis.

Sequence homology assessment and phylogenetic analyses were done to elaborate the distinctness of each species and to initiate research on the immunological basis behind the claim that swamp type buffalo could be more disease-resistant than its riverine counterpart.

“From the cDNA, we translated it into amino acid. We compared the genes of swamp and riverine buffaloes with those of other mammalian species,” Padiernos explained.

Though this study showed that both types of buffaloes including other breeds had high homology, he said, they found out that there are amino acid changes between swamp- and riverine buffalo’s TIM-3, GAL-9 and NRAMP1/2 genes.

This finding provides an insight that the claim that swamp-type water buffalo could be more resistant or tolerant to infectious disease compared to riverine-type water buffalo might be true.

But, he hastened to add, it remains undetermined whether these changes in their genetic material are functionally important. “Thus, further investigation and thorough examination of the other contributing factors to elucidate the differences concerning immunological reactions and providing solution to why a particular animal species is more disease-resistant, tolerant and/or susceptible than its closest relative,” he said.

Selective breeding

“Results of the study showed that the amino acid changes in both types of water buffalo could associate with disease resistance, tolerance and/or susceptibility. If so, this could be a potential gene marker that may be useful in future breeding program for the genetic selection of animals resistant to a particular infection” Padiernos said.

He added that based on the DNA sequence, a gene-based therapeutic protein can be developed to neutralize a specific infection. Through this study, one can establish a system for selective breeding of those buffaloes that are disease-resistant.

But to know if the animal is disease-resistant, its genetic characteristics must be studied and described.

“That’s exactly what we did,” Padiernos said.

The team is now set to conduct further studies in assessing the role of these genes in disease progression in water buffalo.

About the researcher

Ryan Bismark Padiernos who is a graduate of BS Biology from the Central Luzon State University (CLSU), has embarked on several studies on livestock disease prevention, particularly on immunology and virology since joining the PCC as a Science Research Assistant at the Animal Health Unit in 2013. Apart from his research endeavors in PCC, he is also currently involved in the promotion of the Livestock Biotechnology Center of the Department of Agriculture.



The polymerase chain reaction (PCR) is a technology in molecular biology used to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Nematode killing fungi: An alternative control for gastrointestinal parasites

By KHRIZIE EVERT M. PADRE



Parasitic gastrointestinal nematodes cause disease infestation among grazed buffaloes resulting in severe economic losses brought about by poor health, weight and reproduction performance.

This problematic scenario in the livestock industry can now be avoided, as shown in a study titled, “Effect of the Nematophagous Fungi *Duddingtonia flagrans* (*D.flagrans*) as a Biological Control against Common Strongyle Roundworms on Buffaloes (*Bubalus Bubalis*)”.

The research was conducted by a research team led by Toni Rose Barroga of the College of Veterinary Medicine at the University of the Philippines Los Baños, Laguna (UPLB).

Anti-parasitic drugs like anthelmintic is presently being used to control internal parasites. But due to the indiscriminate use of anthelmintics which leads to resistance toward parasites, alternative methods of

control have been introduced. Earlier studies indicated that biological control of internal parasites is a potentially useful alternative control strategy.

Barroga’s research focused on the use of nematode-trapping fungi, particularly *D.flagrans*, for reducing pasture contamination and infection by targeting the free-living larval stages outside the host. Results of this research suggest that *D. flagrans* has the ability to survive the gastrointestinal tract because it can abundantly produce thick-walled resting spores, known as the chlamydo spores, and forms a specialized 3-dimensional sticky network that trap the developing parasite soon after it has been deposited on the ground.

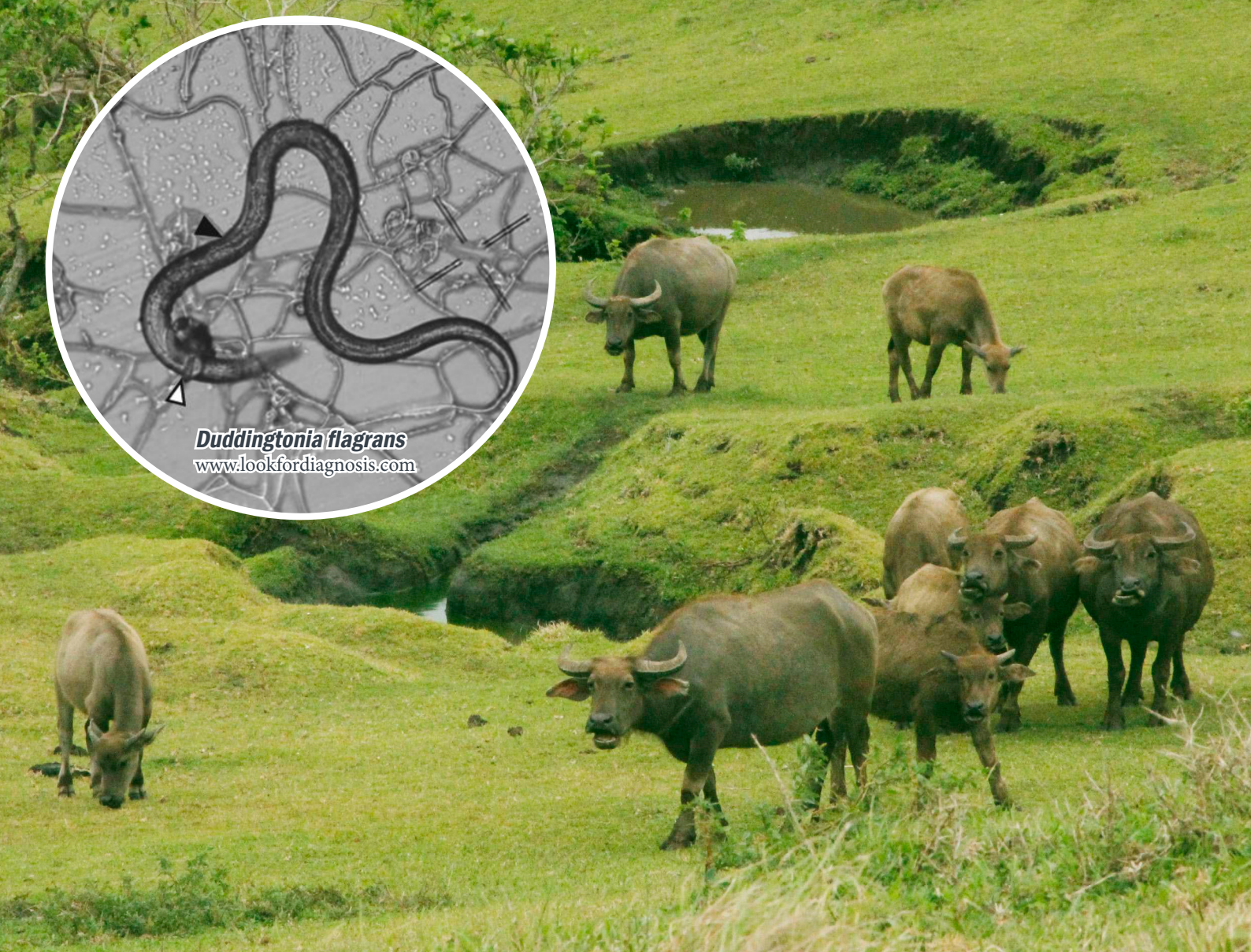
The researcher opted to use three in-vitro experiments: Corn Meal Agar Assay (CMA), Chlamydo spore per Gram (CG) Assay and Chlamydo spore per Egg (CE) Assay while an in-vivo study was conducted to prove the efficacy of *D. flagrans* as a biological anthelmintic administered orally.

The CMA was done to test the predatory activity of *D.flagrans* to strongyle larvae after 48 hours of incubation. Meanwhile, the CG and CE assay were conducted to evaluate the efficacy of *D. flagrans* chlamydo spores on a per gram basis of feces respectively, through determination of percent larval reduction in the fecal larval culture.

To prove the effectiveness of *D. flagrans* as a biological anthelmintic in the animal, increasing doses of chlamydo spore suspension were administered to the experimental buffaloes.

In-vitro experiment using CMA assay showed lower larvae counts on *D.flagrans*-treated group compared with non-treated groups.

In the CG assay, the fecal samples with 2,100 egg/g (EPG) strongyles were treated with increasing doses of chlamydo spores/g feces (100,000, 250,000, and 500,000). There was an 84.39% larval reduction rate after treating with 500,000 chlamydo spore/g



feces.

The use of CE assay, on the other hand, was evaluated with varying treatment ratios (1:100, 1:500 and 1:1000) using the 2,100 EPG feces. It was noted that the 1:500 ratio recorded the highest larval reduction at 78.88%.

In the in-vivo study, after five consecutive days of administering the chlamyospore suspension orally among the fungal treated animals at varying concentrations (50,000, 150,000 and 250,000 chlamydo-spores/kg body weight (BW), the highest larval reduction was recorded at 78.77% with the lowest chlamydo-spores treatment (50,000 chlamydo-spores/kg BW) while the lowest larval reduction was observed at 65% with the highest chlamydo-spores treatment (250,000 chlamydo-spores/kg BW).

The result, according to the researcher, establishes the effectiveness of *D. flagrans* in buffaloes at 50,000 chlamydo-spores/kg BW. The findings also indicate that it is not necessary

to administer higher doses of chlamydo-spore because a low dose would suffice.

The study concludes that using *D. flagrans* as a biological control agent for gastrointestinal parasites is a promising alternative where anthelmintic resistance is a problem.

The researcher recommended further research on the economic impact of using *D. flagrans*, detailing on the financial loss and gains on non-parasitized and parasitized infected herd. More studies should also be conducted to test the ability of *D. flagrans* to reduce larval pasture

contamination.

Barroga was bestowed with the best paper and best presenter awards for this particular study in the recent Philippine Carabao Center's (PCC) R4D In-House Review. She also bagged the UPLB "Best Undergraduate Thesis" in water buffaloes and PSAS "Best Paper in Medicine and Surgery" for the same paper. The members of her team were Dr. Claro N. Mingala, Scientist II of the PCC National Headquarters and Gene Pool, and Therese Marie A. Collantes of UPLB. This study was supported by the PCC.

About the researcher



Dr. Toni Rose M. Barroga is a graduate of Doctor of Veterinary Medicine at University of the Philippines-Los Baños. She currently works as a project staff at the Bureau of Animal Industry under the project "World Organisation for Animal Health (OIE): Stop Transboundary Animal Diseases and Zoonoses (STANDZ) Rabies".



Buffalo milk whey is a good substrate for vinegar production

By CHRISALYN L. MARCELO

Whey, a generally wasted liquid by-product of cheesemaking, is a good substrate for vinegar production, a recent collaborative research study of the Central Luzon State University (CLSU) and the Philippine Carabao Center (PCC) revealed.

According to the joint study, small-scale manufacturers can produce vinegar from whey since it does not require expensive equipment.

The study titled “Utilization of Whey for Vinegar Production” determined the feasibility and the overall characteristics of whey as a substrate for vinegar production.

Specifically, it studied the following: the potential of sweet whey and medium acid whey for commercial vinegar production, the rate of ethanol production as affected by the use of whey for fermentation, the effect of different starters for acetic acid production, the effect of aeration on the rate of acetification and quality of the whey vinegar, the physicochemical and sensory properties of vinegar from whey as compared with commercial vinegar, and the cost of producing the whey vinegar.

The study used a T-test and a three-factor factorial with three replicates to determine the alcoholic and acetic acid fermentation, respectively, and was subjected to analysis of variance at 5% level of significance. To analyze the results of sensory and physicochemical evaluation, the Statistical Package of Social Science (SPSS) and Sirichai Statistical Software were used.

Both sensory and physicochemical evaluations used eight treatments, four for sweet whey and four for medium acid whey, in a combination with two starters (*Acetobacter acetii* and unpasteurized vinegar) and acetic acid fermentation with and without aeration.

Results showed that out of the eight treatments tested, T4 (sweet whey with unpasteurized vinegar and aerated)

and T8 (medium acid whey with unpasteurized vinegar and aerated) are the promising treatments based on the sensory evaluation involving 50 panelists. The two treatments were also found to have acidity levels of 3.51% and 3.55%, respectively, which were higher compared to that of commercial vinegar which has 2.88% of acidity.

In terms of overall acceptability and overall attributes, such as color, aroma, taste, mouth feel, off flavor, turbidity, sourness, and purchase intention, which were evaluated in the study, T8 had the highest scores compared to all treatments including the commercial vinegar.

Nevertheless, both sweet whey and medium acid whey can be used in vinegar production, the study suggests. Other findings of the study are as follows: the rate of ethanol production in medium acid whey is significantly faster compared to sweet acid whey, the unpasteurized vinegar is a better starter medium for expediting the rate of acid production and providing quality in the vinegar, the aeration increased the rate of acetification in the vinegar, and the medium acid whey (aerated) and sweet whey (anaerated) can be produced at a minimal cost. The results conclude that whey is economically feasible as a substrate for vinegar production.

The study recommended that further studies should be conducted on the use of different starters in the whey vinegar production such as *Candida*, *Kluveromyces marxianus* and *Kluveromyces fragilis*. It also recommended studies on the evaluation of the effect of other starters for acetic acid (*Acetobacter pasteurianus*), and

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Did you know?

By CHRISALYN L. MARCELO



Yoghurt has probiotics that can aid proper digestion. Probiotics are microorganisms that provide beneficial effect on the health of human being by improving the intestinal microbial balance. A recent joint study of the Philippine Carabao Center (PCC) and Central Luzon State University (CLSU) proved that yoghurt, which contained the probiotic *Lactobacillus spp.* can tolerate acid in human's gastric pH level, which promotes healthy digestive tract and a more robust immune system. The study showed that out of 10 bacterial strains isolated from the yoghurt produced by PCC, 100% were able to survive at pH3.0, 40% at pH2.5, and 30% at pH 2.0, which proves its important function in maintaining healthier nutrient absorption.

“Evaluation of Probiotic Properties of *Lactobacillus Spp.* Isolated from Plain, Unsweetened Buffalo Milk Yoghurt”

Researchers: Cay Neth A. Callejo, Arman M. Parayao, Federico G. Pineda and Mina P. Abella.

About the researcher

Cay neth Aguirre Callejo is a graduate of Bachelor of Science in Biology at Central Luzon State University. She is the lead of the study titled “Evaluation of Probiotic Properties of *Lactobacillus Spp.* Isolated from Plain, Unsweetened Buffalo Milk Yoghurt”.

Study recommends limited use of tetracycline, sulphonamide in livestock to prevent emergence of bacterial strains with resistance

BY MA. CECILIA C. IRANG

Antibiotic resistance may lead to difficulty of treatment of certain livestock diseases. It may cause some losses for its repeated use since bacteria develop resistance to it.

This was according to a research-based investigation that applies recent protocols in the screening and evaluation of antibiotic resistance. The study titled, "Tetracycline and Sulphonamide-Resistance Genes in Respiratory and Gastrointestinal Bacterial Isolates from Cattle (*Bos taurus*), Goat (*Capra hircus*), and Sheep (*Ovis aries*)" provided information about the presence of resistance of certain bacteria to tetracycline and sulphonamide.

In this study, plasmids carrying resistance genes to tetracycline and sulphonamide were detected. It was conducted to ensure systematic disease screening among animals and to maximize production achieved through a systematic animal health program.

"Since the resistance genes are carried by the plasmids, there may be transfer of these genes from one bacterium to other strains and species of bacteria and there is no concrete information on the extent of antibiotic resistance in animal practice in the Philippines," said Michelle M. Balbin, PCC Science Research Specialist II who conducted the study.

It was funded by the Philippine Carabao Center (PCC) under its Research for Development program.

"We found out through this study that there are genes causing resistance to tetracycline and sulphonamide. These genes are inserting to bacteria, and if the animal got infected with that certain bacteria, and you treat it with tetracycline and sulphonamide, it would be less effective. The bacteria have genes for resistance thus the antibiotics will be less effective," Balbin said.

Tetracycline and sulphonide, Balbin stressed, are two popular antibiotics for treating respiratory diseases. But of the two, tetracycline is more utilized since it

is a long acting drug and it has various forms like oxytetracycline, doxycycline or minocycline. It is also broad spectrum, i.e., that it can cure other diseases like enteritis as long as the causing bacteria are susceptible to these antibiotics.

"These antibiotics are actually effective because they can cure a number of diseases but the problem is that bacteria are already showing resistance so it's time to limit the use of these antibiotics or other drugs and search for alternatives," she explained.

According to the study, there are now some procedures and routines undertaken to counter problems in antibiotic resistance such as tracking the resistance frequency, cohorting, and introduction of new therapeutic approaches. But in addition to this, clinically important bacteria are characterized not only by single drug resistance but also by multiple antibiotic resistance, which is the result of the legacy of past decades of antimicrobial use and misuse.

The rate of antibiotic resistance is significant epidemiologically and ecologically. The consequences of resistance must be considered medically and economically in terms of the responses of animals infected with antibiotic-resistant microorganisms, the researcher said.

As indicated in the study, many of the bacterial pathogens associated with epidemics have evolved into multidrug-resistant (MDR) forms prior to antibiotic use. Bacterial pathogens known to have resistance to some antibiotics include *Acinetobacter baumannii*, *Burkholderia cepacia*, *Campylobacter jejuni*, *Citrobacter freundii*, *Clostridium difficile*, *Enterobacter spp.*, *Enterococcus faecium*, *Enterococcus faecalis*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella spp.*, *Serratia spp.*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Stenotrophomonas maltophilia*, and *Streptococcus pneumoniae*.

Balbin advised that there should be an establishment of data on the extent of

antimicrobial resistance and everyone should be responsible in using these antibiotics. Take note that there already exists Multiple Drug Resistant Bacteria.

Generally, the study was aimed at investigating the tetracycline and sulphonamide-resistance of bacteria related to respiratory and gastrointestinal infections of pigs, small and large ruminants. It specifically sought to evaluate the sensitivity of bacteria and determine the presence of genes that mediate tetracycline and sulphonamide resistance in bacterial isolates of the respiratory and gastrointestinal tract of pigs, small and large ruminants.

Methodologies employed in the study included bacterial strains of six respiratory isolates (*Acinetobacter schindleri*, *Bacillus pumilus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus sciuri* and *Staphylococcus sporosarcina*), in vitro screening for tetracycline and sulphonamide resistance (dilution method), DNA extraction, DNA amplification through Polymerase Chain Reaction, gel electrophoresis of PCR products, and DNA sequencing of tetracycline and sulphonamide resistance genes.

PCR was the technology used to detect plasmid or tetracycline and sulphonamide resistance genes.

Information purposes

"Monitoring of drug resistance is becoming an important component of animal health program. Because of this study, we already have information that certain bacteria have resistance against tetracycline and sulphonamide," Balbin said.

"So the best thing is either to limit its use because there might come a time that it is no longer effective or look for other alternative methods," she added.

She further said that it's already been suspected that bacteria have high resistance against these antibiotics but

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SLA: candidate gene marker for immune response Vs diarrhea in pre-weaning piglets, study shows

BY MA. CECILIA C. IRANG

Diarrhea causes significant economic impact on the success of the swine industry in the country. This is always common in many piglets leading to poor growth rate associated with a low and variable feed intake before and after weaning, the experts said.

Piglets are believed to be susceptible to enteric pathogens and at a younger age or before weaning, the most common disease is diarrhea. It usually occurs after a 3-to 4-day latency period and peaks around one week after weaning. It causes high mortality that affects swine production.

This problem was one of the primary reasons why the Philippine Carabao Center (PCC), thru its Animal Health Unit, conducted a study titled, "Comparative Gene Expression of Swine Leukocyte Antigen (SLA) in Diarrheic and Non-Diarrheic Cases in Pigs (*Sus scrofa*, LARGE WHITE AND NATIVE BREEDS) at Post-Weaning and Pre-Weaning Using Real Time Polymerase Chain Reaction."

The study was under Swine Genomics Project 2 of PCC, Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development (PCAARRD), Bureau of Animal Industry (BAI), and ASBAP.

According to the study, swine leukocyte antigens (SLA), the swine major histocompatibility complex (MHC), codes for a series of extremely polymorphic cell-surface glycoproteins which are critically involved in acute rejection and control of immune responses towards diseases.

It further stated that the SLA region becomes an obvious candidate for marker-assisted selection because of its association and polymorphism which have been reported with variation in the swine immune responses to different diseases.

Studies on the MHC of pigs, the study averred, would be a useful reference for the culling of livestock with altered immune responses and selection of animals with disease resistance which can be associated with production and reproductive performance.

Methodologies used

"We collected blood samples from

20-headpost-weaned piglets that are six to eight-week old. Ten of the animals were diarrheic post-weaned piglets which composed of five native breeds and five Large White and the other 10 were non-diarrheic post-weaned piglets which were represented by five native breeds and five Large White breeds served as negative control," Mary Rose D. Uy, lead researcher of the study, explained.

She also said that 20 pre-weaned piglets (Large White and Native), which were about 2 to 4 weeks of age, were used in the study. The 10 non-diarrheic piglets which were composed of five native breeds and five Large White were used as negative control, while the other 10 comprised of five native breeds and five Large White diarrheic piglets were assigned as positive control.

She further said that they also obtained samples for RNA extraction of SLA type 1 (SLA-1) and SLA type 2 (SLA-2) genes from the blood of diarrheic and non-diarrheic post-weaned and pre-weaned piglets. These samples were reversely transcribed into complementary cDNA (cDNAs) before amplification that applied the primer SLA-1*13XX (which targets the SLA-1 gene with a 217 bp) and SLA-2*w08XX (which recognizes the SLA-2 gene with a 126 bp). Amplified products were analyzed by real-time polymerase chain reaction (qPCR).

Gene expression levels were computed based on comparative CT method. These were tested statistically using Students' T-test.

As revealed in the study, the use of quantitative PCR allowed the researcher to view the entire reaction and product being generated throughout all stages of the reaction. In its simplest and cheapest form, qPCR employed the DNA-binding dye, SYBR green.

"We examined the SLA gene because it is one of the candidate markers in the immune response of pigs in a particular disease," Uy said.

Aside from Uy, the other researchers of the study were Jeffrey Niel P. Aquino, Dr. Gemerlyn G. Garcia, Dr. Claro N. Mingala, Joan Carla F. Sampang, and Dr. Reginaldo V. Abuyuan.

Correlation analysis

Results of the study showed that the expression levels of SLA-1 in post-weaned piglets were slightly in piglets experiencing diarrhea, however, the statistical result showed no significant differences. In SLA-2 genotype, the expression levels between diarrheic and non-diarrheic post-weaned piglets had also no significant differences.

On the other hand, in pre-weaned piglets expression level of SLA-1 in diarrheic Native and Large White were significantly higher than that of the non-diarrheic piglets ($P < 0.0399$). SLA-2 expression level was not significantly different in non-diarrheic and diarrheic native and Large White piglets.

Correlation analysis showed negative relationship in the expression of SLA-1 and SLA-2 in diarrheic and non-diarrheic Native and Large White piglets. The data gathered demonstrated that SLA-1 and SLA-2 accompany diarrhea in Native and Large White piglets in terms of their CT values which were significantly different ($P < 0.0094$).

"SLA type 1 apparently mediates or triggers immune responses of pigs to diarrhea at pre-weaning," Uy declared.

The study aimed at quantifying and determining the difference in the expression of SLA-1 and SLA-2 gene expression in a course of diarrheic and non-diarrheic conditions in post-weaned and pre-weaned piglets.

"Results of the study showed that pre-weaned piglets are seen to have higher expression of SLA gene compared to the post-weaned. Higher expression happens when piglet has been infected, which means it has immune response against certain disease and it is expressed more in the pre-weaning piglets in several folds," she added.

Importance

"The data that were gathered will serve as a reference in improving the health status of farm animals and in increasing productivity. Information regarding SLA genes and their products are useful tools in deciding for the elimination of livestock with altered or weak immune responses

(Continued on next page)

Assessing semen... (From page 3)

based on kinematic attributes, which can be further correlated with their capacitation, fertilization, and reproduction ability.

With this final identification of good and bad freezer donors, the research team was essentially assured of the availability of quality buffalo bulls and semen, which are critical to the Agency's National Genetic Improvement and Cryobanking Program in water buffaloes.

CASA standardization

Another plan of the research team is to have an association study wherein they will use the AI and pregnancy records as bases in the selection of fertility-rated bulls. They will collect semen of these bulls to determine the sperm combination thru CASA and they need to come up with standard values of CASA for buffalo semen.

"We will start gathering semen from 50 bulls to run under CASA to get the standard values for buffalo. In the study, we only selected and ran samples from 6 bulls and it is not enough to develop a standard that is why we need to conduct another project

for standardization of CASA," Maylem expounded.

She added: "We will select bulls that have high AI efficiency rate and we will assess their sperm thru CASA and associate them with the newly-arrived bulls to develop a standard. It is not possible to use all bulls with good quality semen because our standard values will become high, that is why they should all be homogenous."

Meanwhile, Dr. Eufrocina Atabay, one of the researchers in the study and head of the PCC reproductive biotechnology unit, said that fertility is equal to high quality semen, perfect timing of ovulation, and good genetics. She said they are still verifying if the good attribute of CASA is directly associated with fertility rate.

"Our initial results are not yet conclusive. We still need verification in the field and further study as there are other aspects or factors that can affect the over-all fertility. It is not only the semen but also the female's egg that should be studied," Atabay said.

Other members of the research study

team are Dr. Edwin Atabay, Dr. Emma Venturina, Dr. Flocerfida Aquino, and Dr. Lerma Ocampo.

"We cannot declare that CASA can really enhance fertility rate because sometimes, inside the female tract, there are factors to consider. No matter how good the quality of the semen is, the female system has its own way of rejecting it. But for me, since we are not sure if the egg will be fertilized, it would always be wise to start with a very good quality semen," Atabay said.

As of today, there are also CASAs installed in the PCC at CLSU, Digdig, Carrangalan, PCC at UPLB, and PCC at CMU for semen production and hopefully, for the standardization and harmonization of researches.

About the researcher

A graduate of BS Biology, Excel Rio S. Maylem is currently a Science Research Specialist II at the PCC Reproductive Biotechnology Unit. Her researches are focused on semen quality (CASA), HSP70 gene expression, IVM-IVF, and Fixed-Time AI.

Buffalo milk whey...

(From page 8)

exploring the possibility of using whey as substrate for distilled ethanol production. The study also suggests that buying of compressor may also be considered to lessen the cost of aerated vinegar production. Compressor is a mechanical device that is used to compress and pressurize air in the vinegar during aeration process.

The study was conducted by Rica Nianne D. Perez, Catherine Jane M. Callanga, Ma. Princess D. Veneracion, Jessica M. Rustia, and Mina P. Abella. It was co-implemented by PCC at CLSU and CLSU and was funded by the former.

About the researchers

The researchers are newly graduate students with the degree Bachelor of Science in Food Technology at Central Luzon State University. Jessica M. Rustia and Mina P. Abella served as their advisers.

Study recommends... (From page 9)

there is limited documentation especially as they relate to Philippine livestock.

"It is a must to examine the bacteria whether it is resistant or susceptible to antibiotics. This kind of practice is usually done in a diagnostic laboratory. Samples are taken and antibiotic sensitivity test is conducted. This examination provides the appropriate antibiotics to be used, but of course in emergency cases we can't do that. And, that is the importance of establishing data for this matter" she said.

The study recommended further researches that will test the sensitivity of potential pathogens to different kinds of antibiotics as a rational basis for the administration of treatment.

Further works directed toward the molecular detection of antibiotic resistance gene in potential pathogens coming from different body systems are also encouraged in order to come up with complete information on the extent of tetracycline and sulphonamide and other antimicrobial resistance in the veterinary practice or in a particular group of animals.

About the researcher

A veterinarian by profession, Dr. Michelle M. Balbin currently works as a science research specialist II at the PCC Animal Health and Biosafety Unit. Her research focus is on surveillance and monitoring of infectious diseases in ruminants; and in development of different laboratory protocols for the detection of diseases.

SLA: candidate gene... (From page 9)

and in the selection of animals with disease resistance," she explained.

Further studies on the identification of pathogen-causing diarrhea in piglets and its association on immune responses of MHC classical I genes should be made. Moreover, regulation mechanisms involving SLA entail elaborate investigation and a significantly higher number of

experimental animals is needed.

About the researcher

Mary Rose D. Uy, a graduate of BS and MS in Biology, currently works as a Science Research Specialist under the Swine Genomics Project housed at the PCC Animal Health and Biosafety Laboratory unit. She is actively involved in molecular-based researches dealing with disease resistance in swine and water buffaloes.

OPINION



‘Better by the Bundle’

ERIC P. PALACPAC, PhD
PCC National R4D Coordinator

The word “bundle” is often encountered in the world of marketing wherein a combination of products are sold as a package deal. Buying a laptop computer bundle for example may include (aside from the laptop) goodies like a printer, a flash drive, and a laptop case. It is thus a very good marketing strategy that entices the consumer to buy by the bundle because more often the cost of each item in a bundle may be more expensive if bought separately.

In the context of research, the practice of “bundling” is also increasingly becoming popular as a strategy in providing integrative solutions to a common research problem. This time, a bundle consists of individual research projects (with each project specializing on a particular field) that are packaged as one research “program” as they are inextricably linked to one another. Here, an individual project is considered as an integral part of a holistic solution and it cannot exist or cannot be understood without reference to the latter. In other words, the old cliché, “the whole is greater than the sum of its parts”, is at the very core of a “bundled” research.

“Bundling” offers opportunities for “interfacing” (a very popular byword nowadays for us at PCC) among researchers, hence, for interdisciplinary approaches in addressing a particular concern.

In dairy buffaloes for example, a common problem is how to increase the volume of milk produced. Normally, a researcher

may look at a nutritional intervention while another researcher may provide breeding management strategies. The usual practice is the two conduct researches in their respective fields on their own or independent from one another. This is typical in a disciplinary type of research. While it may provide answers to the research problem, more often, they are fragmented solutions that are only temporary and cannot be sustained. A more holistic, bundled research that looks into improving nutritional deficiencies in buffaloes coupled with novel breeding strategies may provide more impact on increasing their milk production. This is the beauty of “bundling” and the benefit of “interfacing”.

Recently, a “bundling” approach was adopted by PCC in its submission of a research program proposal to PCAARRD. The proposal is an interface of animal nutrition, reproduction, health, milk quality, and enterprise development in its quest to increase the volumes of milk produced by dairy buffaloes in Nueva Ecija and in San Agustin, Isabela. It was eventually approved with a budget of close to Php64 million over a three-year period. While the said research is still to formally start in early 2016, the PCC is already adopting the “bundling” approach in its other research efforts particularly those for implementation by its 13 regional centers. And it’s just logical – for many of the problems in the carabao sector are very much interrelated, hence, integrative solutions are inevitable.

R4D Highlights[®]

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