

R4D *Highlights*



Researchers present 20 new discoveries in PCC's in-house review; top works cited

By MERVALYN TOMAS

Twenty research results that aim to address industry research gaps in water buffaloes and other livestock were presented and evaluated during the annual R4D in-house review of PCC.

The review, held last June 27-28 at the PCC National Headquarters and Gene Pool, centered on five disciplines: biosafety, product development and management, animal genomics, cryopreservation and techniques, and socio-economic aspects of the Carabao Development Program implementation.

“The annual R4D In-House Review serves as a learning platform for PCC researchers to share significant findings of their on-going or completed researches in their respective

disciplines. Presenting research results in a review format is a way of validating and improving further research perspectives,” Dr. Eric Palacpac, research for development (R4D) coordinator, said.

The presented papers are comprised of undergraduate student thesis, internally and externally funded researches, and commissioned researches.

Dr. Palacpac said the review underscored

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compelling researches for the advancement of PCC's mandate and for the continuous development of the livestock sector.

A panel of evaluators judged the papers accordingly as they were presented.

The study on a sperm-specific protein in water buffaloes won this year's "Best Paper".

Titled "Molecular Characterization and Comparison of Phospholipase C Zeta (PLCZeta) Between Swamp type (*Bubalus carabanensis*) and Riverine type (*Bubalus bubalis*) Buffaloes," the "Best Paper" garnered 91 percent approval rating from the panel. A team composed of Dr. Eufrocina Atabay, Dr. Edwin Atabay, Dr. Claro Mingala, Dr. Emma Venturina, Dr. Rafael Fissore, and Roseline Tadeo composed the research team for this work.

The evaluators recognized the importance of PLCZeta, which is widely known to induce oocyte activation following fertilization, being finally characterized in water buffaloes.

Tadeo, Science Research Assistant at the PCC Reproduction and Physiology Unit who presented the research output, also won as the "Best Presenter".

On the other hand, the study on "Molecular Detection of Tetracycline-resistance Genes in Respiratory Bacterial Isolates of Small and Large Ruminants" by Allan Jeffrey Francia, Gemerlyn Garcia, Dr. Michelle Balbin, and Dr. Mingala won the "Best Undergraduate Student Research Paper".

Dr. Florencia Charito Sebastian, University of the Philippines-Diliman Extension Program (UPDEPP) director; Prof. Janne Calica of UPDEPP; and Anna Reylene Montes, PCC-UPLB Science Research Specialist presented the PCC-commissioned researches.

The resource panelists who were tapped for the two-day event were veterinarian Dr. Jose Arceo Bautista of the Animal Dairy Sciences Cluster-UPLB, Dr. Virginia Venturina, Dean of the College of Veterinary Science and Medicine of the Central Luzon State University (CLSU), and CLSU College of Agriculture Professor Zosimo Battad II.

Dr. Palacpac, encouraged scientists and researchers from the PCC network, as well as the student-participants, to strive harder to create meaningful and relevant researches.



R4D Coordinator Dr. Eric Palacpac (left) poses with one of the resource panelists Professor Zosimo Battad II (center) of CLSU College of Agriculture, and the Best Presenter Roseline Tadeo (right) during the awarding ceremony.



Roseline Tadeo, research assistant of the study titled "Molecular Characterization and Comparison of Phospholipase C Zeta (PLCZeta) between Swamp type (*Bubalis carabanensis*) and Riverine type (*Bubalus bubalis*) Buffaloes uses Polymerase Chain Reaction (PCR) to amplify PLCZeta genes of swamp and Riverine types of water buffalo.

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Study recommends limited use of antibiotics in livestock to prevent emergence of bacterial strains with resistance

By MA. CECILIA IRANG



Antibiotic sensitivity test result of *Acinetobacter schindleri*.

Antibiotic resistance, caused by the repeated use of it, may lead to difficulty of treatment in certain livestock diseases. This poses economic losses in the industry.

This was according to an empirical investigation that applies recent protocols in the screening and evaluation of antibiotic resistance among livestock. 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 antibiotics such as tetracycline and sulphonamide.

In this study, plasmids carrying resistance genes to tetracycline and sulphonamide were detected. It was conducted to ensure accurate disease screening among animals and to maximize production 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 Dr. Michelle M. Balbin, PCC Science Research Specialist II, the author of the study.

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

“We found out that there are genes causing resistance to tetracycline and sulphonamide. If the animal is infected with that certain bacteria carrying those genes, treatment with tetracycline and sulphonamide would be less effective ” Balbin said.

Tetracycline and sulphonamide, Dr. 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 such 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 to it. This is why we recommend limiting the use of these antibiotics or other drugs and opt for alternatives instead,” 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 antimicrobial use and misuse in the past decades.

“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,” Dr. Balbin 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 in 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 recommends that there should be an establishment of data on the extent of antimicrobial resistance and caution should be exercised when using antibiotics. Take note, she said, that there is already such a thing as MDR Bacteria.

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Use of routine diagnostic tool can detect suspected cases of bovine herpesvirus-1 on imported cattle, study says

By MA. CECILIA IRANG

Infectious bovine rhinotracheitis (IBR) or bovine herpesvirus 1 (BoHV-1) is recognized as one of the economically important diseases and a highly contagious infectious respiratory disease that infects ruminants primarily cattle, the experts said.

If afflicted by it, the adult cows will have a severe and prolonged decrease in milk yield, will reduce their fertility, and have abortions.

The disease is characterized by clinical signs of the upper respiratory tract, such as a (muco) purulent nasal discharge, hyperaemia of the muzzle (red nose disease) and by conjunctivitis. Other signs of general illness include fever, depression, inappetance, and salivation.

So far, there has been no accurate report of cases involving this disease in the Philippines. But, in order to help prove or disprove the presence of the disease in the country and also help in

the surveillance, control program and biosecurity measure for BOHV-1, the study titled “Optimization of Different Polymerase Chain Reaction (PCR) Techniques for Sensitive Detection of Bovine Herpesvirus 1” was conducted by a research team from the Philippine Carabao Center (PCC).

“If ever we will be importing animals from other countries, BOHV-1 is one of the diseases that we can screen using different PCR protocols for us to execute necessary biosecurity measures. In this way, we will be able to prevent this disease from entering the country. We will have a diagnostic test that can be used later,” Dr. Michelle M. Balbin, PCC Science Research Specialist II and a member of the research team, said.

Disease surveillance and monitoring, she added, can be an aid in avoiding economic losses in the local dairy industry.

She further said various tests have been issued by the OIE Terrestrial Manual

for BOHV-1, which includes serological techniques like virus neutralization test and Enzyme-Linked Immunosorbent Assay (ELISA), virus isolation, and recently PCR which is increasingly used in routine diagnosis. Of these three techniques, PCR is the easiest technique so far, according to Dr. Balbin.

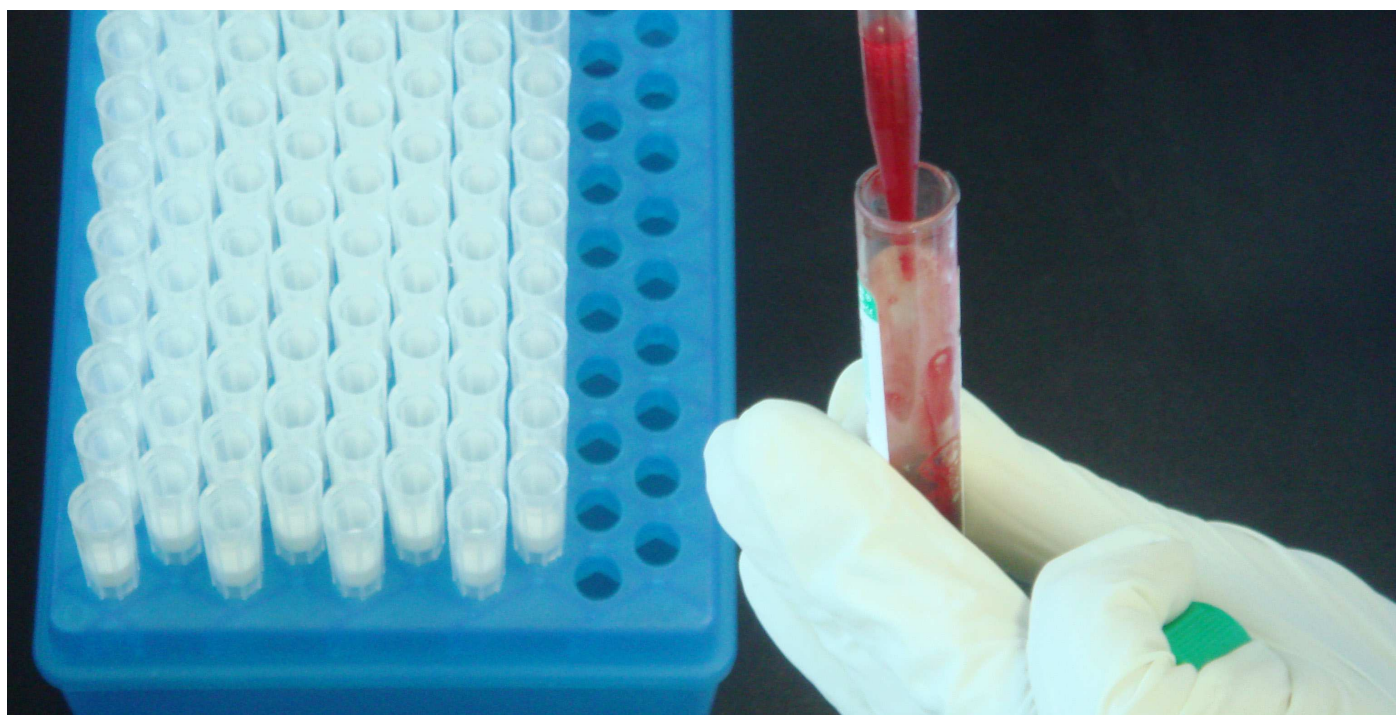
Different PCR protocols such as single-step PCR, nested-PCR and real-time PCR were optimized to detect BoHV-1. They can also be used in different diagnostic laboratories, the researchers said.

According to Dr. Charles Patrick Davis of eMedicineHealth, PCR is a technique that is used to amplify trace amounts of DNA (and in some instances, ribonucleic acid or RNA) located in or on almost any liquid or surface where DNA strands may be deposited.

Methodologies employed

In 2013, the research team of Dr.

(Continued on page 6)



One of the methodologies employed in the study was blood collection of selected cattle in farms where BoHV-1 was reported.

PCC reveals first-ever research on buffalo sperm-specific protein

BY MERVALYN TOMAS



PCC reveals the first-ever study done on buffalo PLCZeta, a protein found only on sperms that induces oocyte activation.

The study titled “Molecular Characterization and Comparison of Phospholipase C Zeta (PLCZeta) between Swamp type (*Bubalis carabanesis*) and Riverine type (*Bubalus bubalis*) buffaloes” shows characterization and comparison between the PLCZeta gene of swamp and riverine types of buffaloes.

“This study is foundational to a deeper understanding of buffalo PLCZeta’s biological mechanisms and ultimate realization of its potential benefits in livestock production,” Roseline Tadeo, one of the researchers, said.

The study is under the project

called “Screening for Sperm-factor (Phospholipase C-zeta) by Molecular Technique as a Novel Biomarker of Bull Fertility for Genetic Improvement in Water Buffaloes” led by Dr. Eufrocina P. Atabay, Scientist 1 at the PCC Reproduction and Physiology Section.

What is PLCZeta?

The PCC research team, composed of Dr. Atabay, Tadeo, Dr. Edwin Atabay, Emma Venturina, Raphael Fissore, and Dr. Claro Mingala, explained that PLCZeta is a novel sperm-specific protein that acts as a major physiological agent in fertilization.

Tadeo said PLCZeta’s function is to induce series of calcium oscillations, which activates the egg and triggers embryo development.

According to Dr. Atabay, PLCZeta is the main protein to induce calcium oscillation during mammalian fertilization and embryonic development. Calcium oscillation is calcium mobilization from outside the cell to the cytoplasm.

Findings and their significance

The researchers found that PLCZeta of the two breeds are almost similar in molecular structure.

On the other hand, they were able to identify a difference in their amino acid sequences.

This variation may affect the PLCZeta functions in the specific breed such as oocyte activation, fertilization and development of embryo, according to

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Study recommends...

(From page 3)

Generally, the study 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.

“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” Dr. Balbin said.

Procedures employed in the study included the use of 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.

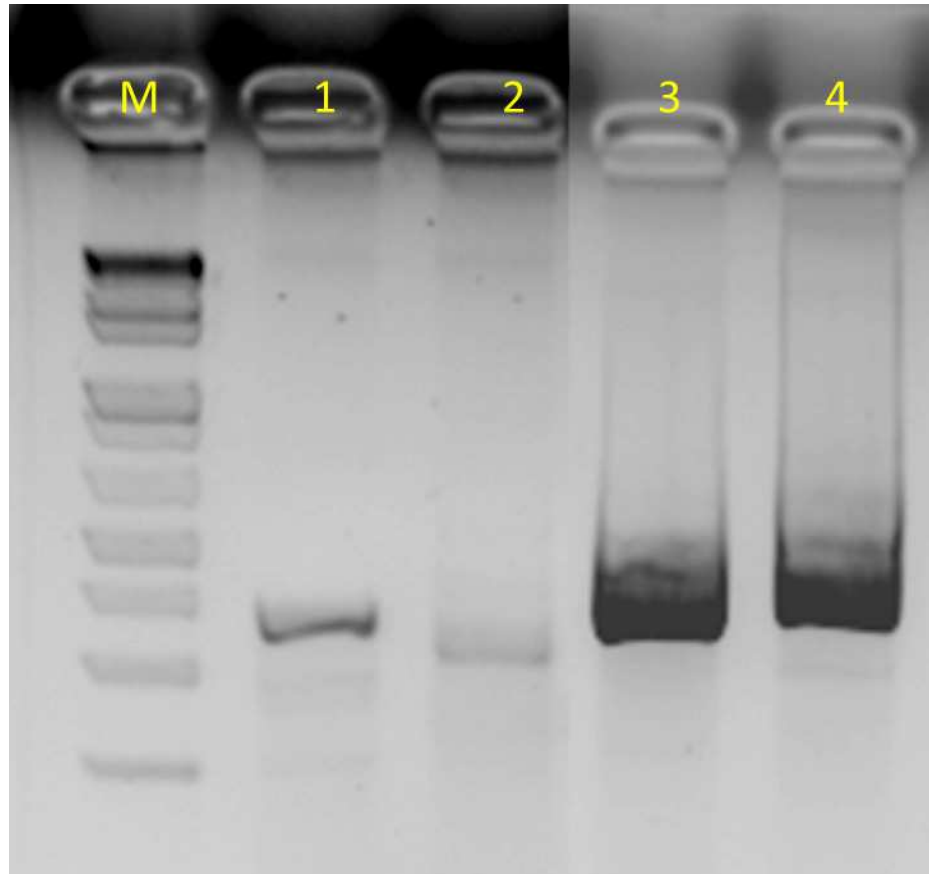
PCR was the technology used to detect plasmid or tetracycline and sulphonamide resistance genes.

The study recommends that further researches should be done to test the sensitivity of potential pathogens to different kinds of antibiotics as a rational basis for the administration of treatment.

Further investigation on the molecular detection of antibiotic-resistance gene in potential pathogens coming from different body systems is 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.

Use of routine diagnostic tool ...

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As indicated in the study, lines 1- 4 are IBR positive controls. The second PCR has an amplification size of 385 bp (lines 1 and 2), while the first PCR has 478 bp (lines 3 and 4) expected amplification size.

Fumitoshi Saito reported detection of antibodies against BoHV-1 and bovine parainfluenza virus-3 in farms in South Luzon Area.

“Whole blood samples were collected from 40 selected cattle in farms wherein BoHV-1 was reported. Twenty of the cattle were identified as seropositive of BOHV-1 while the rest are seronegative,” Dr. Balbin explained.

Primers amplifying the gB viral surface was used to optimize protocols for single-step PCR, nested-PCR and real-time PCR in the detection of BoHV-1 in dairy cattle. Synthetic plasmid was used as positive control for the detection of BoHV-1. Using the different PCR protocols, BoHV-1 was not detected in the samples.

“Since the history of the animals were unknown and the farm has history of importing animals, we could deduce that these seropositive cattle were previously vaccinated with the existing BOHV-1 vaccines but may not carry the BoHV-1 pathogen,” Dr. Balbin said.

Aside from Dr. Balbin, the other

researchers of the study were Dr. Claro Mingala, Ron Rian Manuela, Dr. Lawrence Belotindos, and Jonathan Lazaro.

Conclusions and recommendations

The study concluded that the use of an optimized PCR protocol to detect the gB gene of BoHV-1 in dairy cattle blood assumes the possibility of BoHV-1 being absent in the Philippines.

This protocol can be used as a routine diagnostic tool for imported cattle and confirm suspected cases.

However, for surveillance purposes, increasing the number of animals is highly recommended. The use of different BoHV-1 (eg. gE gene), the study also suggests, should be ventured.

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.

Whey, a cheese making by-product, can be developed into a sports drink, studies show

By CHRISALYN MARCELO



Whey is a liquid by-product in cheese making and is usually dumped because it has no value. It is the greenish translucent liquid which remains after the curds are formed and drained in cheese production.

Research studies, however, indicated that whey has important nutrients like lactose, protein, minerals and vitamins. Its protein is considered as high-quality in terms of nutrition, flavor, functionality and value necessary to create great-tasting sports product formulations.

Previous literature has also proven that whey has the “highest biological value and protein efficiency ratio and contains all of the essential amino acids required by the body for good health.” It can also deliver the benefits of “burning more fat while retaining muscle,” “increasing lean muscle” and “enhancing physical performance” over other protein sources consumed after exercise.

Thus, whey, can also be processed into beneficial products like sports drink and whey vinegar, among others.

Sports drink

The study titled, “Development of Lactase - Treated Isotonic Drink from Whey”, explored the possibility of using whey to develop a sports drink (isotonic beverage) from lactase-treated whey. It was conducted by Central Luzon State University (CLSU) Bachelor of Science in Food Technology students Lynnden Castillo, Charlene Mae Lazo and Rafael Amurao under the supervision of Dr. Alma De Leon of the College of Home Science and Industry and Mina P. Abella of the Philippine Carabao Center (PCC).

Specifically, the researchers aimed to determine the efficiency of lactase enzyme in reducing the lactose content of the whey, the physico-chemical properties, sensory characteristics and nutritional composition of the isotonic whey drink, as well as the percent yield and production

cost of the by-product when made into a sports drink.

Lactase is an enzyme that degrades or reduces the lactose (milk sugar) in whey. It is used to increase the solubility and digestibility of the sports drink, thus, making it more ideal for consumers who are lactose intolerant.

In the development of isotonic drink from lactase-treated whey, the following were considered: hydrolysis of whey; optimization of parameters such as temperature, reaction time and concentration; lactase determination of glucose in hydrolyzed whey; preparation of isotonic drink from hydrolyzed whey; and product evaluation.

In the process of hydrolysis, acid whey was treated with lactase to hydrolyze the lactose in the whey. Hydrolysis of lactose was conducted by adding lactase to the whey at a rate of 2% and at 23oC for 1 hour. Results of the study showed, at the optimum condition, lactase has the capability to reduce the lactose content by 92%.

Hydrolyzed whey, together with other ingredients was well blended and the pH of the mixture was adjusted to 3.8 to 4.3 using 10% citric acid. Thereafter, the drink was heat-treated to a temperature of 92oC for 2 minutes and hot-filled into PET bottles and sealed. Two treatments were used in the study: one with mango, lemon and orange flavors and another one with no flavor is the control treatment.

Meanwhile, the physico-chemical characteristics, microbiological quality and nutritional information of the isotonic drink were also determined in the study. In the sensory evaluation, the researchers used key performance indicators (KPI) such as overall acceptability, product ratings such as liking, and just-about-right on specific attributes such as color, aroma, taste and mouthfeel.

Based on the overall results, the following highlight the findings:

No viscosity was observed on all treatments, though the highest total soluble solid (TSS) was found in the orange-flavored isotonic drink, which is not significantly different from the lemon-flavored drink.

Nutritional analysis also revealed that mango flavored whey drink has total calories of 73.34 with a carbohydrate content at 7.20%, which qualify it as an isotonic drink (a drink is said to be isotonic if its carbohydrate content is within 4%-8%). Furthermore, the isotonic whey drink has low fat content at 1.93% and a protein content at 6.79%, which makes it a good source of amino acids.

Other than that, the mango flavor was the most liked sample, followed by lemon and orange flavors. Likewise, the flavor with the highest purchase intention by customers was found to be the mango -flavored isotonic drink.

And based on all sensory evaluation attributes conducted in the study, majority of the panelists rated all treatments as “just-about-right” in terms of its color, aroma, taste and mouthfeel.

Meanwhile, during the storage at ambient temperature, the total aerobic plate count and yeasts and molds count of isotonic whey drink are found to be within the FDA standards up until the 4th week of storage. The salmonella, coliform and *E.coli* counts in the isotonic whey drink are all found negative, which meets the FDA microbiological standards for ready -to-drink beverages.

All treatments, based on the penalty analysis, can still be improved by increasing the levels of fruity flavor, sweetness, mouthfeel, sourness, overall flavor and aroma, according to the study.

The cost of production per 250ml isotonic drink was at Php 26.09.

The range of purchase decision from the 50 panelists, meanwhile, was found to be at 3.62 to 4.06. It meant that consumers will “probably” and “definitely” buy the developed isotonic drink when it becomes available in the commercial market.

About the researcher

Mina Abella, senior science research specialist, is one of the advisers in the collaborative study of PCC and CLSU titled “Development of Lactase-treated Isotonic Drink from Whey.” She currently works as the head of the Carabao Enterprise Development Section at the PCC National Headquarters and Gene Pool.



Study establishes agronomic characteristics, nutrient composition of 'Pakchong 1' when grown under Philippine condition

BY CHRISSALYN MARCELO

Pakchong 1, an improved grass developed by Dr. Krailas Kiyothong of Thailand's Department of Livestock Development (DLD), was studied by a group of researchers from the Central Luzon State University and the Philippine Carabao Center (PCC), to determine its agronomic characteristics and nutrient composition under Philippine condition.

Pakchong 1 is a cross of ordinary Napier grass (*Pennisetum purpureum*) and pearl millet (*Pennisetum glaucum*). It was introduced by Dr. Kiyothong to PCC last 2014 before it was studied by the latter last April 2015 to March 2016.

Titled "Agronomic Characteristics and Nutrient Composition of Pakchong 1 Using Vermicast", the study used a 3x3 factorial design to determine its above stated objective using three treatments namely inorganic fertilizer, vermicast and inorganic fertilizer with vermicast.

Dr. Daniel L. Aquino, one of the researchers, said they used 12 quadrants, measuring 2mx6m per quadrant, in

planting Pakchong 1 cuttings in PCC's pasture area in the Science City of Muñoz in Nueva Ecija.

"For treatments 1, 2, and 3, we used about 1, 016.5 kg of inorganic fertilizer, 360 bags of vermicast, and 5, 082.5 of vermicast with 1, 016 kg inorganic fertilizer, respectively, in a hectare per year, in the study," Dr. Aquino explained.

He added they used the nutrient depletion of the grass as the basis of the rate of their application for the inorganic fertilizer treatment while they used 1:5 bag ratio as basis for the application of the vermicast with inorganic fertilizer treatment. They also used three cutting intervals such as 45, 52, and 60 days after planting.

The study showed the following results:

The use of inorganic fertilizer gave the highest herbage yield of Pakchong 1, which is about 500, 000 kg per hectare per year, compared to the vermicast (303, 750 kg) and inorganic fertilizer with vermicast (455, 000 kg), during the first cycle.

The use of inorganic fertilizer also gave the highest dry matter yield of 109, 722 kg per hectare per year on the average compared to the vermicast (44, 722 kg) and inorganic fertilizer with vermicast (98, 799 kg).

The use of vermicast, gave the highest herbage yield of 202, 000 kg per hectare per year on the average compared to inorganic fertilizer (194, 917 kg) and inorganic fertilizer with vermicast (192, 458 kg) during the second cycle.

Furthermore, the study showed that the use of inorganic fertilizer with vermicast gave the highest crude protein of 11.32% compared to the inorganic fertilizer (10.59%) and vermicast (8.29%). Its herbage yield and dry matter yield are quite similar to the use of inorganic fertilizer alone, too, according to the study.

Other results indicated that, Pakchong 1 had: (1) dry matter ranging from 18%-20%; (2) neutral detergent fiber (NDF) ranging from 58%-61%; (3) ash content ranging from 8%-12%; and (4) crude protein ranging from 7-11%.

The study recommended the following for further studies:

- (1) Continually monitor the agronomic characteristics and nutrient composition of Pakchong 1 to cover one production cycle to include wet and dry seasons;
- (2) Perform a complete soil analysis to determine the soil components of the pasture area;
- (3) Consider regular monitoring on the occurrence of pathogens or pests in the area;
- (4) Determine the nutrients digestibility/ degradability to access the salient nutrients that are available to animals; and finally,
- (5) Determine the economic cost of production and feeding of Pakchong 1 to dairy animals.

Other researchers who conducted the study were Dr. Ernesto Garillo and Ivan Carl Dela Rosa of CLSU and Phoebe Lhyndia Llantada of PCC.

About the researcher

Dr. Daniel Aquino is a technical expert on animal nutrition and is currently the Center Director of PCC at CLSU. The said center is one of the 12 centers of PCC nationwide. It is located in Science City of Muñoz, Nueva Ecija.

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

By MA. CECILIA IRANG



Mary Rose Uy, lead researcher of the study, collects blood samples of six to eight-week old piglets to be analyzed.

Diarrhea causes significant economic losses in the country's swine industry. This is particularly common in piglets leading to their poor growth rate associated with low and variable feed intake before and after weaning, experts say.

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 three-to four-day latency period and peaks around one week after weaning. It leads to high mortality, significantly damaging an otherwise economically viable swine production.

This problem was one of the primary reasons why 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 is under the Swine Genomics Project 2 of PCC, Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development, and Bureau of Animal Industry.

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 affirmed, can be a useful reference for selection of animals with disease resistance which can be associated with reproductive performance.

Methodologies used

"We collected blood samples from 20-head post-weaned piglets that are six to eight-week old. Ten of the animals were diarrheic post-weaned piglets, which are composed of five Native breeds and five Large White (positive control) and the other 10 were non-diarrheic post-weaned piglets which were represented by five Native breeds and five Large White breeds, which served as negative control," Mary Rose D. Uy, lead researcher of the study, explained.

She also said that 20 pre-weaned piglets, 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 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 expressed 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

(Continued on page 10)

PCC reveals... (From page 5)



The researcher prepares reagent to be used in amplifying the PLCZeta gene of Swamp and Riverine types of water buffalo.

the researchers.

“Variations may play a role in gene and protein expression, physiological mechanisms, and biological functions of the two breeds,” the researchers said.

They explained that the variations of PLCZeta sequences identified in the study can be correlated with semen quality and fertility to enable genomic selection, which can hasten genetic improvement not only in water buffaloes but also in other livestock.

Tadeo explained the few variations may influence biological functions in water buffalo fertilization.

“This will also provide a biomarker for screening the bull’s fertilizing ability to enhance reproductive efficiency and productivity in water buffaloes,” Tadeo said.

On the other hand, the researchers concluded that water buffalo PLCZeta has high sequence identity with that of the cattle and other domestic species.

Method

In conducting the study, the researchers collected water buffalo semen samples from each breed of the Philippine carabao (draft type) and the Italian carabao (dairy type). The sperms were separated from the seminal fluid. Total ribonucleic acid (RNA), a molecule essential in the expression of genes, was isolated from sperm pellets and reverse-transcribed.

The PLCZ1 cDNA was amplified and then subjected to gel electrophoresis, a technique commonly used in the laboratory to separate charged molecules, like DNA and RNA, according to size. Subsequently, it was submitted to a firm, First Base Malaysia, for sequencing.

About the researcher

Roseline Tadeo, a biologist who graduated from Central Luzon State University works as a science research assistant at the PCC Reproduction and Physiology Section. She is currently working under the project called Screening for Sperm-factor (Phospholipase C-zeta) by Molecular Technique as a Novel Biomarker of Bull Fertility for Genetic Improvement in Water Buffaloes.

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

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 was aimed at quantifying SLA-1 and SLA-2 gene expression in a course of diarrheic and non-diarrheic conditions in post-weaned and pre-weaned piglets; and determining the difference in the expression of SLA genes (SLA-1 and SLA-2) in diarrheic conditions of 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. This gene is being expressed if, for example, it is higher, it 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.

Significance of the study

“The data that were gathered will serve as a reference in improving the health status of farm animals and in increasing productivity,” 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 examined, the researchers recommend. Moreover, regulation mechanisms involving SLA entail elaborate investigation and a significantly higher number of experimental animals should be studied, they concluded.

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.

Fermented corn grain by-product can increase buffalo milk production, income, study says

By MERVALYN TOMAS



Corn distillers dried grains with soluble (DDGS) are packed for the specific treatments.

Dairy carabaos' milk can be increased by feeding them with corn distillers dried grains added with solubles (DDGS), a study by PCC showed.

Higher concentration of DDGS fed to the dairy carabaos resulted in the production of more milk by the animals, the researchers said. Dr. Daniel Aquino, Center Director of PCC at Central Luzon State University (CLSU), Dr. Irene Domingo, Department Chair of the CLSU Department of Animal Science and Cherry Ann Alvaran, CLSU student, authored the said study.

They said the carabaos fed with as much as 50 percent of DDGS yielded higher average milk production compared to those fed with only 25 percent DDGS concentration.

The study was funded by PCC in collaboration with a private DDGS supplier. The DDGS, explained the researchers, is a by-product of corn grain fermentation in the ethanol industry in the United States. It is used for dairy cows as it is a very good source of protein for the animal.

Since it is dry, it is the most easily

transported by-product in the production of ethanol. It can also be easily stored under reasonable conditions for considerable period of time. In the study, all the buffaloes fed with 50 percent of DDGS, as a replacement of the commercial dairy concentrates, were noted to have increased their milk production after 120 days. Those fed with only 25 percent DDGS had lower average milk production and the animals which were not fed with DDGS registered the lowest milk production during the entire duration of the study.

"DDGS is an excellent, low -cost alternative feed ingredient produced in large quantities by the dry-grind fuel ethanol industry," the researchers averred.

They cited the US grains council saying that this feed material has high energy, mid-protein, and high bioavailable phosphorus content that make it a very attractive, partial replacement for some of the more expensive energy sources such as corn, protein-rich diet like soybean meal, and phosphorus such as monocalcium phosphate used in animal feeds.

They further said that when DDGS is added to properly formulate feed mix, it

resulted in the animal's excellent health and performance and in their food product quality. These attributes have made DDGS one of the popular feed ingredients for use in animal feeds around the world.

Other than increase in milk production, an increase in the sales of milk yielded from those fed with DDGS compared to those which were not was also noted.

The use of DDGS in the animal's feed, was also found to be a big money saver.

"The expense was only Php8.58 for a kilogram of milk produced by the carabaos fed with 50 percent compared with those fed with 25 percent DDGS, with Php9.74, and Php11.49 for those without DDGS," the researchers said.

To get more conclusive result, the researchers recommended longer feeding of DDGS to lactating buffaloes covering one or more cycles of milk production.

About the researcher

Cherry Ann P. Alvaran is a graduate of BS Agriculture major in Animal Science. She was a thesis advisee of Dr. Daniel Aquino, Center Director of Philippine Carabao Center at Central Luzon State University.

OPINION

Making the “transition”

ERIC P. PALACPAC, PhD

PCC National R4D Coordinator

It is the time of the year when the Christian world is in a festive mood in anticipation of the Yuletide Season. For those who are employed in the government and the private sectors, the much awaited Christmas “bonus” is also on their minds, in anticipation of the expenses that go with the season’s celebration (well, that’s the legacy of commercialism that clouds the true meaning of Christmas...just saying).

Here at the Philippine Carabao Center (PCC), it is also the period of reckoning, whereby we review our accomplishments vis-à-vis the targets set for the year, as reflected in the agency’s Major Final Output (MFO) Accountability Report Card (MARC). In the government bureaucracy, the MARC is the yardstick of performance in all its agencies and instrumentalities. As a form of recognition and incentive to individual agencies and their employees, the government has also established a performance-based bonus (PBB) system, which is anchored on the MARC. In other words, those who are MARC-compliant (at least 90% accomplishment of the MFOs), will receive the appropriate PBB incentive.

Early this year, the PCC had the distinction of being the first agency under the Department of Agriculture (DA), which was granted with the much coveted PBB for hitting an average of more than 90% of its physical targets and fund utilization for the previous year. Of course, that is a cause for celebration. The PCC has demonstrated to all that it is one of the most competent and productive agencies in the DA. As employees of PCC, it is a reason for us to be happy, proud, and motivated. We are thus, looking forward to another shot at PBB, as we review our MARC for 2016. But in doing so, I have this nagging question in mind that I just like to come out, i.e., **how is our performance as a research and development (R&D) agency?**

I asked this because the PCC is touted as the DA’s leading livestock R&D

agency with all its research outputs and accomplishments over the years, yet, nowhere in the PCC’s MARC can you see R&D performance parameters or indicators. Instead, what we report in our MARC are technical support services with emphasis on production, market development, and extension. We do so because this is how the Department of Budget and Management (DBM) categorized us and provided budget for our existence as an attached agency of the DA. The PCC’s top management has exerted efforts in the past lobbying at the DBM to rationalize the functions of PCC as a “true” R&D agency, with appropriate revisions of its MFOs. Still, the DBM has not yet given us the green light.

For many personnel of PCC particularly those stationed in the regional centers, the call from the top management for them to engage more actively in R&D is often met with anxiety. While many are eager to engage in R&D, they are also pressured to prioritize on and deliver the MFOs, which, as stated, are heavy on the production, marketing, and extension. In the past, we introduced the concept of “operations research” in an attempt to bridge the gap, but only a few regional centers have practiced so.

In light of the above premises, the PCC should reassert its position and manifest again its previous intention at the level of the DBM to be rationalized as an R&D agency for water buffalo (and other livestock for that matter), in the mold of the Philippine Rice Research Institute (PhilRice). The latter has a MARC that looks very simple and straightforward with R&D performance indicators that represent almost 50% of its activities and budget allocation.

With the recent creation of the PCC’s Research & Development Division (RDD), a DBM-approved unit under the leadership of Dr. Annabelle Sarabia, I hope we can make the “transitions” in perspectives, priorities, MFOs, and performance evaluation in the near future. That is actually my Christmas wish for the agency.

R4D Highlights®

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For comments and suggestions, please write to the Editor-in-Chief in this mailing address:

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