Philipp J Vet Anim Sci 2019 45(1):48-57

EGG PRODUCTION PERFORMANCE OF IMPROVED PHILIPPINE MALLARD DUCKS (*Anas platyrhynchos*) FED DIETS SUPPLEMENTED WITH FRESH TRICHANTHERA (*Trichanthera gigantea*) LEAVES

Janine I. Berdos[1], Ernesto A. Martin[2], Oliver F. Celestino[2] and Ella Joyce S. Paragas[2]

ABSTRACT

The nutritional values and feeding trial of fresh Trichanthera (*Trichanthera gigantea*) leaves for Improved Philippine Mallard Duck (IPMD) layers were examined. Proximate, calcium, phosphorus and energy contents of Trichanthera were analyzed. Its effects on egg production, fertility and hatchability were analyzed when used as part of a ration of IPMD on early lay performance. For the feeding trial, a total of 108 IPMD were randomly assigned in three treatments following Completely Randomized Design (CRD); each treatment had three replication with 12 ducks (2 drakes and 10 ducks) per replicate. The treatments were: without Trichanthera, with 50 g Trichanthera/duck/day and with 100 g Trichanthera/duck/day. When expressed on dry matter (DM) basis, Trichanthera contained 93.30% DM, 19.59% crude protein, 11.89% crude fiber, 2.33% crude fat, 20.15% ash, 4.47% calcium, 0.25% phosphorus and 2,310 kcal ME/kg. The combined intake of feed and Trichanthera was higher ($P<0.01$) for IPMD fed 100 g Trichanthera/duck/day and had numerically higher egg production (85.88%) than their counterparts. Gain in weight of the IPMD during the seven-week period was not influenced ($P>0.05$) by Trichanthera feeding. Egg quality, egg classification, fertility and hatchability of eggs, and quality of hatchling were not affected by Trichanthera. Numerically, higher IOFC (Php 47.43/duck) was attained from IPMD fed 100 g of Trichanthera/ duck/day.

Key words: dietary supplement, Improved Philippine Mallard Duck, income over feed cost, litter-floor and Trichanthera

INTRODUCTION

Eggs are the most important product from the Philippine duck industry. Ducks are next to chicken in terms of economic importance as source of eggs as well as meat. Still, problems including insufficient space for free-range operations, quality breeder ducks, unstable supply of ready-to-lay pullets, high cost of feeds, fluctuating prices of eggs and limited research studies being conducted on duck raising are regarded as constraints to the

[1]Tarlac Agricultural University, Camiling, Tarlac 2306, [2]Central Luzon State University, Science City of Muñoz, Nueva Ecija (e-mail: janine.berdos@gmail.com).

industry (Agriculture, 2016). These problems explain why the volume of production has been declining for the past five years, which dropped to an average of 5.42 percent (PCAARRD, 2016).

Since there is a significant increase in the domestic utilization of duck products, the prospect for the development of the industry is promising. With this, Improved Philippine Mallard Duck (IPMD) was developed, a product of continuous selection and breeding of the traditional Pateros duck (Parungao, 2017). However, the development of IPMD necessitates the development of feed and feeding system to attain consistent egg production performance and product quality.

Traditionally, supplementation of protein to ducks during egg production is practiced. Unfortunately, sources of supplement such as snails and small shrimps have become scarce. In this regard, there is a need to explore for potential locally available plants as protein source to lessen feed cost and increase the profit of duck raisers (Lacayanga, 2015). Trichanthera (*Trichanthera gigantea*), also known as Nacedero, can be considered for this purpose. It is a fodder tree that adapts well in tropical conditions, grows easily between plantation crops. Its protein content ranges from 17% to 22% on DM basis and has high calcium content compared to other fodder trees (Rosales, 1997; Garcia *et al*., 2008). Therefore, the study was conducted to assess the proximate composition, calcium, phosphorus and energy value of Trichanthera and its effects on egg production, fertility and hatchability when used as part of the ration of IPMD on early lay performance.

MATERIALS AND METHODS

A total of 108 25-week old IPMD with an average weight of 1.50 kg were used in the study. They were randomly assigned to three experimental treatments following Completely Randomized Design (CRD). Each treatment had three replicates with ten ducks and two drakes for each replicate. The experimental treatments were: 1) Basal diet (BD) only, 2) BD + 50 g fresh Trichanthera leaves/duck/day and 3) BD +100 g fresh Trichanthera leaves /duck/day.

Green Trichantera leaves were gathered using pruning shear. After harvest, the leaves were immediately chopped and offered together with the basal diet. The basal diet (Table 1) was least-cost formulated to contain the recommended nutrients for laying ducks for optimal performance (Datuin, 2003). The diet was in mash form and was mixed using a rotary-type electric feed mixer.

Dried samples of 150 g Trichanthera were placed in separate zip-lock plastic bags marked and sealed and were sent by courier service to UPLB for proximate, energy, calcium and phosphorus analyses.

To calculate the overall ADG and uniformity, IPMD were weighed individually at d 0 (start of the experiment) and at d 49 (end of the experiment). The uniformity of the IPMD was determined at the initial and final day of the study. It was calculated by getting the weight of the ducks plus or minus 10% of the mean body weight over the number of ducks weighed multiplied by 100. Total feed offered and feed refusal at the end of each period was also weighed. Additionally, feed spillages from the drinkers and feeders were recovered to calculate for overall ADFI. To calculate for overall ADFI of Trichanthera, leaves left at the end of the day were weighed. FCR was calculated by dividing ADFI with the egg mass. Egg mass was calculated by multiplying egg weight by hen-day egg production.

Table 1. Ingredient and nutrient composition (as fed basis) of duck layer diet.

Item	Price/kg (Php)	Basal Diet
Ingredient, %		
Yellow Corn	17.00	53.27
Soybean meal, USHP	40.00	26.82
Rice bran, D1	13.00	4.21
Molasses, coarse	18.00	4.41
Palm oil, refined	65.00	1.00
Limestone, coarse	7.00	4.00
Limestone, fine	6.00	3.81
Salt	7.00	0.35
Monodicalcium phosphate	30.00	1.39
Vitamin premix[1]	975.00	0.03
Mineral premix[2]	109.00	0.15
Choline chloride	93.00	0.10
DL-Methionine	224.00	0.11
L-lysine	75.00	0.30
Ethoxyquin	325.00	0.02
Toxin binder	19.00	0.02
Total	100.00	100.00
Calculated composition, %		
ME (kcal/kg)	--	2700
CP	--	18.00
Crude fiber	--	4.00
Crude fat	--	2.42
Met	--	0.40
Met+Cys	--	0.71
Lys	--	1.22
Thr	--	0.67
Trp	--	0.21
Ca	--	3.50
P, available	--	0.40
Diet Cost/kg, Php	--	24.53

[1]The vitamin premix provided the following quantities of vitamins per kg of complete diet: vit. A, 65,000,000 IU; vit. D3, 5,000,000 IU; vit. E, 100,000 mg; vit K3, 10,000 mg; vit. B1, 10,000 mg; vit. B2, 27,000 mg; vit. B6, 15,000 mg; vit. B12, 200 mg; niacin, 200,000 mg; folic acid, 5,000 mg; pantothenic acid, 60,000 mg; and biotin, 1,000 mg.

[2]The mineral premix provided the following quantities of minerals per kg of complete diet: iron, 80,000 mg, copper, 10,000 mg, zinc, 80,000 mg, manganese, 70,000 mg, cobalt, 200 mg, selenium, 200 mg and iodine, 800 mg.

Egg composition and egg quality were estimated based on hen-day egg production, albumen height, yolk color score and weight, shell weight and albumen weight. A total of 3,463 eggs were collected every 6:00 am and weighed to estimate the egg weight. A total of 72 eggs were evaluated for egg composition and egg quality in the last two weeks (post-peak production) of the study. Furthermore, the fertility of eggs was determined during the first candling (9th day of incubation) using a candler. A second candling (18th day of incubation) was also done before hatchability. The newly hatched ducklings were individually classified into normal or with abnormalities (e.g. navel condition and physical deformities).

The cost per kilogram of Trichanthera was based on the time devoted in gathering of leaves. Income over feed cost (IOFC) was calculated as the difference of the total sale value of eggs and cost of feeds consumed per hen-housed.

Data were analyzed using ANOVA of STAR (Statistical Tool for Agricultural Research). The least-significant differences (LSD) test was used to determine significant differences between treatment means at $P = 0.05$.

RESULTS AND DISCUSSION

The analyzed proximate, energy, calcium, and phosphorus values (Table 2) generally typified the chemical composition of Trichanthera and were in most cases in agreement with those in the literature (Table 3). Results indicate that the crude protein and calcium in Trichanthera were of main interest in the study where it was expected to influence eggshell synthesis by IPMD. The amount of crude protein was high and consistent with those in the literature (Jaya *et al*., 2008). It is also regarded that Trichanthera contains a high amount of essential amino acids and that most of the crude protein is true protein (Rosales 1996; Rosales, 1997). On the other hand, the crude fiber and gross energy values were lower. It

Table 2. Proximate, energy, calcium and phosphorus content of Trichanthera leaves on DM basis.

Item	Amount
Proximate, %	
Moisture	6.70
Ash	20.15
Crude protein	19.59
Crude fiber	11.89
Crude fat	2.33
Nitrogen free extract	39.34
Calcium, %	4.47
Total phosphorus, %	0.25
Energy, kcal/kg	
Gross energy	3665
Metabolizable energy	2310

Table 3. Published proximate or chemical composition of Trichanthera.

Parameters	A[1]	B[2]	C[3]
Proximate, %			
Dry matter	20.00-26.90	78.90	88.44
Moisture	-	-	11.56
Crude protein	17.90-22.50	23.90	18.21
Crude fiber	-	23.80	12.50
Ether extract	-	2.50	2.66
Ash	-	24.30	21.80
Nitrogen-free extract	-	25.50	-
Ether extract	-	2.50	2.66

[1]Tricanthera values as fed; [2, 3]-Tricanthera values in DM basis

A-Rosales (1997); B- Sarwatt *et al*. (2003); C- Jaya *et al*. (2008)

is recognized that these differences can be attributed to samples used for analysis, which had been influenced by season, variety, fertilization, irrigation, soil type and environment (Oelberg, 1956; Adebayo *et al*., 2017) where the Trichanthera was grown.

There were no significant (P>0.05) differences among groups regarding the production parameters, except for daily feed intake in which lower value (P<0.01) was observed for IPMD in the control group and IPMD offered 50g /duck/day compared with their counterpart (Table 4). Final weight of birds fed without Trichanthera was significantly different (P=0.01) with those fed with Trichanthera. Uniformity was comparable among groups.

Results indicate that the differences in feed intake could be explained per unit change in Trichanthera intake (McDonald *et al*., 2010). It was also considered that *ad libitum* feeding influenced the feed consumption of IPMD under litter-floor management, yet it did not deteriorate egg production and egg size (Avens *et al*., 1979).

The significantly lower final weight of the IPMD fed with 100 g Trichanthera was apparently associated with other factors like physiological since IPMD were layers, thus, they were not required to be overfed. These ducks had the highest egg production, with satisfactory FCR. As such, factor from Trichanthera can be ruled out, especially so that their intake for Trichanthera was low. However, uniformity of body weight of not less than 70% is regarded as satisfactory in poultry (Welten, 2016).

There were no differences in quality and composition of eggs between IPMD fed with and without Trichanthera. Statistical analyses show that the kind of ration did not significantly (P>0.05) influence egg classification (Table 5). This finding indicated that Trichanthera as part of the IPMD ration did not influence the quality of yolk, albumen and eggshell, irrespective of the feeding level. Likewise, Trichanthera was not a factor for yolk color. Trichanthera had a moderate amount of CP and a very high amount of calcium. It was evident that at the level of intake of Trichanthera, the IPMD had egg material and shell synthesis including deposition of yolk pigment comparable without Trichanthera.

Results indicate that there were no toxic factors from Trichanthera that negated egg size. It was noteworthy though that there was a preponderance for large size eggs, irrespective of the IPMD ration. Furthermore, dietary factors such as energy, methionine and linoleic

Table 4. Comparative production performance, body weights and uniformity of IPMD with and without fresh Trichanthera leaves.

Item	Treatment			*P*-Value
	Without	50g/ duck/ day	100g/ duck/ day	
Production performance				
HD egg production, %	77.92	78.01	85.88	0.13
ADFI + Trichanthera ADFI, g/day	171.07[b]	170.28[b]	180.00[a]	0.01
FCR (Feeds + Trichanthera), g/day	3.93	5.24	3.99	0.43
Egg weight, g	68.96	66.92	68.09	0.30
Egg mass, g	54.09	52.92	58.89	0.15
Body weights and uniformity				
Initial weight, g	1478	1422	1611	0.07
Initial weight uniformity ,%	66.67	77.78	55.56	0.70
Final weight, g	1533[a]	1437[b]	1398[b]	0.01
Final weight uniformity, %	100.00	77.78	88.89	0.58
Gain in weight, g/day	9.23	7.98	2.31	0.75

[ab] Within a row, means without a common superscript differ ($P<0.05$).

which affected egg size, were comparable among diets (March and MacMillan, 1989; Ruan *et al*., 2015; Fouad *et al*., 2016).

The fertility and hatchability of eggs were not affected ($P>0.05$) by the inclusion of Trichanthera (Table 6). There were no differences in the classification of ducklings regarding their quality and weight. The present data indicate that the egg fertility of the IPMD exceeded 80% for mallard ducks (PCARRD, 2006), irrespective of their ration. Hatchability can be influenced by breed or strain of ducks, temperature and humidity and turning of eggs during incubation (King'ori, 2011). The breed can be an utmost considered factor such that heavy breeds like ducks, were less efficient in the deposition of thiamine into the egg which was necessary for the embryonic development and hatchability (Wilson, 1997). However, Trichanthera did not affect embryonic mortality and hatchability yet it was unclear what particular factor influenced the low hatchability in the present study.

The results present a high percentage of good quality hatchlings indicating the quality of their ration. Good quality chicks hatched from eggs weighed at least 40 g, but good uniformity could be obtained on the average from eggs weighing 48 g-50 g (for breeder standards). It can also be determined by having clear and bright eyes, homogenous size, alert and free from leg deformities with a clean navel (Cazaban, 2005). Their weight was comparable to the initial weight of Pekin (47.05 g) and Muscovy (46.39 g) ducklings (Rashid *et al*., 2009).

The IPMD fed with and without Trichanthera did not differ in eggs produced. However, IPMD fed with 100 g/duck/day had the highest sale value of eggs but had the highest feed consumed. Overall, the highest income over feed cost (IOFC) can be derived

Table 5. Comparative quality, composition and classification of egg produced from IPMD with and without fresh Trichanthera leaves.

Item	Treatment			*P*-Value
	Without	50g/ duck/ day	100g/ duck/ day	
Egg quality and composition				
Albumen height, mm	8.32	8.51	8.34	0.83
Albumen weight, g	40.50	40.42	40.00	0.96
Yolk weight, g	23.75	23.08	21.75	0.14
Yolk color score, DSM units	6.67	7.63	7.25	0.10
Eggshell weight, g	7.79	7.50	7.33	0.22
Egg weight, g	72.05	71.00	69.09	0.48
Egg classification, %				
No weight (<47 g)	0.20	0.10	0.00	0.56
Small (48-56 g)	1.02	2.00	2.65	0.40
Medium (57-65 g)	18.78	29.36	22.21	0.31
Large (66-74 g)	57.55	55.96	55.21	0.84
Extra-large (75-83 g)	21.94	12.49	19.56	0.40
Jumbo (84≥)	0.51	0.10	0.37	0.28

Table 6. Comparative reproductive performance of IPMD with and without fresh Trichanthera leaves.

Item	Treatment			*P*-Value
	Without	50g/ duck/ day	100g/ duck/ day	
Fertility and hatchability, %				
Fertility	89.66	95.17	88.92	0.09
Hatchability	69.00	72.35	72.20	0.90
Classification and weight of ducklings				
Good quality hatchlings, %	83.63	100.00	95.52	0.38
Body Weight, g	44.38	44.13	45.12	0.63

from eggs produced by IPMD fed with 100 g Trichanthera with a value of Php 47.43 per duck compared with its counterparts (Table 7). The results indicated that Trichanthera is affecting egg synthesis to attain optimal sale value of eggs. Predictably, the feed cost increased with decreasing Trichanthera, but there is an economic advantage of including Trichanthera to a diet which in turn be profitable when it will be adopted into a larger farm scale. However, it was only based on the production for the first seven weeks from the point of lay.

In conclusion, Trichanthera leaves contained moderately high crude protein, high

Table 7. Income over feed cost of IPMD with and without fresh Trichanthera leaves.

Item	Treatment			*P*-Value
	Without	50g/ duck/ day	100g/ duck/ day	
Eggs produced[1], pcs.	38	39	42	0.32
Sale value of eggs[2], Php	247.00	253.50	273.00	0.21
Feed consumed[3], kg	8.41[a]	7.96[b]	8.60[a]	<0.00
Feeds consumed, PhP	206.39[a]	195.35[b]	211.01[a]	<0.00
Trichanthera consumed[4], kg		1.30	1.22	
Trichanthera consumed, Php		15.56	14.56	
Feeds and Trichanthera consumed, Php	206.39[b]	210.92[b]	225.57[a]	<0.00
IOFC, PhP	40.61	42.58	47.43	0.11

[1]Average egg production per bird for 7 weeks.
[2]Suggested retail price per egg is based on Paul's Balut Industry as of January 2018. Price per fresh egg is PhP 6.50.
[3]Average feed consumed per bird for 7 weeks and diet cost per kg is PhP 24.53.
[4]Trichanthera cost per kg is PhP 12.00 but may vary depending on the number of chopping hours of Trichanthera.
[ab] Within a row, means without a common superscript differ ($P<0.05$).

calcium and low in energy. Trichanthera as part of the ration supported satisfactorily the production and reproduction performance of IPMD. Moreover, feeding Trichanthera increased IOFC.

ACKNOWLEDGMENT

We acknowledge the financial assistance given by CHED K to 12 Transition Program for this study. Likewise, to PCAARRD for partly supporting the work in terms of the ducks used, the College of Agriculture Center of Excellence of CLSU for the Duck Research Facility and Paul Balut's Industry for the incubator used during fertility and hatchability tests.

REFERENCES

Adebayo A, Akintoye A, Shokalu O and Olatunji T. 2017. Soil chemical properties and growth response of *Moringa oleifeira* to different sources and rates of organic and NPK fertilizers. *Int J Recycl Org Waste Agricult* (6):281-287.

Agriculture. 2016. Native Duck Raising. https://www.pressreader.com/philippines/agriculture/20160801/281702614088367.

Avens J, Athearn R and Mcneal J. 1979. Egg production and efficiency of food conversion of khaki campbell ducks under different management systems. *Br Pout Sci* 21 (5): 333-337.

Cazaban C. 2005. Day-Old Chick Quality. http://www.thepoultrysite.com/focus/contents/ceva/ onlinebulletins/ob_2005/article-no1-july-05.pdf.

Datuin J. 2003. Energy and protein requirements of Philippine mallard ducks. Unpublished doctoral dissertation. Central Luzon State University (CLSU), Science City of Muñoz, Nueva Ecija.

Fouad M, Ruan D, Lin Y, Zheng C, Zhang H, Chen W, Wang S, Xia W and Li Y. 2016. Effects of dietary methionine on performance, egg quality and glutathione redox system in egg-laying ducks. *Br Poult Sci* 57(6):818-823.

Garcia D, Medina M, Cova L, Clavero T, Torres A, Perdomo D and Santos O. 2008. Integral evaluation of fodder resources for ruminants in Trujillo State, Venezuela. *Rev Fac Agron* 26(4):555-582.

Jaya A, Soriano L, Villador D, Intong R and Carpentero B. 2008. Utilization of Madre de agua (*Trichanthera gigantea* var. *guianensis*) leaf meal as feed for growing-finishing pigs. *Philipp J Vet Anim Sci* 34(2):117-126.

King'ori A. 2011. Review of the Factors That influence egg fertility and hatchability in poultry. *Int J Poult Sci* 10(6):483-492.

Lacayanga C. 2015. Effects of different levels of madre de agua, lead tree and horseradish fresh leaf as partial replacement of feeds on egg production performance of mallard duck. *Int J Sci Basic Appl Re*s 24(3):71-85.

March B and Macmillan C. 1989. Linoleic acid as a mediator of egg size. *Poult Sci* 69:634-63.

McDonald P, Edwards R, Greeham J, Morgan C, Sinclair L and Wilkinson R. 2010. Animal Nutrition. 7th edition. Pearson Education.

Oelberg, K. 1956. Factors affecting the nutritive value of range forage. *J Range Manag* 9: 220-225.

Parungao A. 2017. Improved Philippine Mallard Duck development promotion and utilization in building rural enterprises. Retrieved from http://www.pcaarrd.dost.gov.ph/home/portal/index.php/quick-information-dispatch/2970-itik-pinas-development-promotion-and-utilization-in-building-rural-enterprises.

PCARRD. 2006. The Philippines recommends for duck egg production. 3rd edition. Los Baños, Laguna : Philippine Council for Agriculture, Forestry and Natural Resources Research and Development 2006.

PCAARRD. 2016. PCAARRD and BAI to boost local duck industry. http://www.pcaarrd.dost.gov.ph/home/portal/index.php/quick-information-dispatch/2045-pcaarrd-and-bai-to-boost-local-duck-industry.

Rashid M, Kawsar M, Rashid M, Miah M and Howlider M. 2009. Fertility and hatchability of pekin and muscovy duck eggs and performance of their ducklings. *Progress Agric* 20(1-2):93-98.

Rosales M. 1996. *In vitro* assessment of the nutritive value of mixtures of leaves from tropical fodder trees. (Published doctoral dissertation). University of Oxford, England, United Kingdom.

Rosales M. 1997. *Trichanthera gigantea* (Humboldt & Bonpland.) Nees: A Review. *Livestock Res Rural Dev* 9(4).

Ruan D, Lin Y, Chen W, Wang S, Xia W, Fouad A and Zheng C. 2015. Effects of rice bran on performance, egg quality, oxidative status, yolk fatty acid composition, and fatty acid metabolism-related gene expression in laying ducks. *Poult Sci* 94:2944-2951.

Sarwatt S, Laswai G and Ubwe R. 2003. Evaluation of the potential of *Trichanthera gigantea* as a source of nutrients for rabbit diets under small-holder production system in Tanzania. Retrieved from http://lrrd.cipav.org.co/lrrd15/11/sarw1511.htm.

Welten P. 2016. Fleshing important in optimal broiler breeding. https://www.poultryworld.net.

Wilson H. 1997. Effects of maternal nutrition on hatchability. *Poult Sci* 6:134-143.

2nd National Conference on Food, Environment, Engineering and Technology (2nd NCFEET)
JOURNAL ON FOOD, ENVIRONMENT, ENGINEERING AND TECHNOLOGY
ISSN 2599-4123 VOLUME 2, NO. 1, 2019

LOW-COST LIQUID ANIONIC SURFACTANTS IN MASTITIS DETECTION FOR SMALL-HOLD DAIRY BUFFALO FARMERS

YANCY O. WAMINAL[1,*], JENNIFER E. GANGAN[2]
*ORCID NO.: 0000-0002-5440-8533
[1]*Department of Animal Science, College of Agriculture and Forestry, Tarlac Agricultural University, Camiling, Tarlac 2306, Philippines*
[2]*College of Agriculture, Pangasinan State University- San Carlos Campus*
Waminal0830@gmail.com

Abstract

The study was conducted to evaluate the effect, consistency, as well as the economic viability of using liquid anionic surfactants (LAS) in mastitis detection in order to provide cleaner milk. A total of forty two (42) milk samples from lactating dairy buffaloes at two (2) different farm locations were used in the study. The experiment was laid out following the Completely Randomized Design with three replications.

Statistical findings showed that CMT and Liquid anionic surfactant with 30 percent to 50 percent dilution level have more or less similar Mastitis detection capability. Based on the economic viability, preparation of liquid anionic surfactants with 30% dilution level is cheaper in terms of cost per sample and cost of producing 1 gallon of test solution when compared to CMT reagent. The result of the laboratory analysis for somatic cell counting also confirmed that Liquid anionic surfactants from 30% to 50% dilution level registered very close results to CMT in detecting types and grades of mastitis infection from dairy buffaloes. Conclusively, 30 % dilution rate can substitute to CMT with regards of mastitis detection for easier and accessible use of the reagent in a shortest possible time at a low-cost.

Keywords: *CMT, liquid anionic surfactant, low-cost, lactating buffalo, mastitis*

Introduction

Mastitis is the most prevalent disease afflicting dairy animals and destroys the milk quality. The teats' exposure to infectious bacterial agents such as *Staphylococcus aureus, Streptococcus agalactiae*, *Streptococcus uberis*; Coliform bacteria: *Escherichia coli, Klebsiella, Aerobacter aerogens*, *Pseudomonas aeruginosa, Coryne bacterium pyogene;* and Mycoplasma organisms frequently leads to infection.

The CMT or California Mastitis Test, the WMT Wisconsin Mastitis Test, Modified Whiteside Test, Somatic Cell Count or SCC and other laboratory methods of mastitis detection are very effective tests but are not readily available in the market. It also requires technical expertise and not affordable to ordinary farmers. Hence, the study focus on developing a low-cost mastitis test which does not require expensive solutions and very practical in detecting mastitis infection at the shortest possible time

The study was conducted to evaluate the effect, consistency, and the economic viability of different levels of liquid anionic surfactants in mastitis detection. A total of 42 milk samples from 11 lactating water buffaloes regardless of age and weight were used. Collection of milk sample for laboratory analysis was done before milk testing using the Calfornia Mastitis Test (CMT) and liquid anionic surfactants (LAS).

JOURNAL ON FOOD, ENVIRONMENT, ENGINEERING AND TECHNOLOGY
VOLUME 2, NO. 1, 2019 ISSN 2599-4123

Methodology

Milk testing in different farm locations and milking parlor infrastructure was done early in the morning and late in the afternoon in accordance with the protocol of the dairy laboratory. About 30 ml of milk was drawn from four quarters of the udder into the centrifuge tube. The tubes were labeled with the animal number and teat location and immediately store in the ice chest. The animal number, calving/kidding date, milk yield per day, and the result of CMT and LAS test for each quarter using Mastitis chart were recorded in the sheet provided by the Philippine Carabao Center Dairy laboratory.

Milk samples collected from each experimental animal was submitted to the Philippine Carabao Center Dairy Laboratory- Animal Breeding and Genomics Section for Somatic Cell Count Analysis using Cytometry. The experiment was laid out following the Completely Randomized Design with three replications.

Results and Discussion

Table 1 showed that the use of liquid anionic surfactant at different inclusion levels (30%-50%) is very helpful in detecting sub clinical and clinical mastitis in dairy buffaloes and can be substitute to CMT.

The result of the laboratory analysis for somatic cell count conducted by the Philippine Carabao Center Dairy Laboratory confirmed the tabulated results gathered during the macroscopic evaluation. As per results of the Somatic Cell Counting, different levels of liquid anionic surfactant register very close results to CMT in terms of detecting types and grades of mastitis infection. By justification, the anionic surface-acting agent akyl anyl sulfonate, is an active ingredient that can be found in surfactants and in CMT reagent have the same action in the deoxyribonucleic acid (DNA) of the cell. Both Liquid Anionic Surfactant and California Mastitis Test (CMT) reagent dissolves or disrupts the outer cell wall and the nuclear cell wall of any leukocyte, which are primarily fat (detergent dissolves fat). DNA is released from the nuclei. The DNA will get together to form a stringy mass. As the number of leukocytes increase, the amount of gel formation will increase in a linear fashion (CMT, 2011).

Based on the economic viability, 30% liquid anionic surfactant solution is cheaper in terms of cost of preparation and it is more readily available in the market nationwide compared to California Mastitis Test reagent.

Conclusion

The results concluded that mastitis detection using liquid anionic surfactant is an easy, cheap and a helpful tool in controlling the spread of mastitis by early detection and treatment of the disease. Preparation of the solution is very simple, the component of the mixtures are readily available and affordable. The use of the solution (liquid anionic surfactant) will prevent waste of milk through early detection of the infection that will lead to increased milk production especially among small hold and commercial scale of dairy industries.

Table 1. Test results of the macroscopic evaluation and economic viability using CMT and different dilution levels of liquid anionic surfactant.

Dilution levels	Negative	Trace	Mastitic Grade 1	Mastitic Grade 2	Mastitic Grade 3	Total	Cost per sample (PhP)
T1 – California Mastitis Test Reagent (Control)	34	2	4	1	1	42	1.96
T2 – 30% liquid anionic surfactant + 70% distilled water	34	3	3	1	1	42	0.70
T3 - 40% liquid anionic surfactant + 60% distilled water	31	5	4	1	1	42	0.91
T4 - 50% liquid anionic surfactant + 50% distilled water	32	4	4	1	1	42	1.05

References

[FAO] Food and Agriculture Organization. 2014. Impact of mastitis in small scale dairy production systems. Animal Production and Health Working Paper. No.13 Rome

Halasa T, and Osterus O. (2007). Economic effects of bovine mastitis and mastitis management. Vet Q. 29: 18–31.

Jones GM, and Bailey TL. (2010). Understanding the Basics of Mastitis. Virginia Cooperative Extension. 46–53.

Khan MZ, and Khan A. (2006). Basic factors of mastitis in dairy animals. Pakistan Vet J. 26: 2048

Ogola H, and Shitandi A. (2007). Effect of mastitis on raw milk composition quality. J. Dairy Science. 8: 237–242

Scanned with CamScanner

integrity Research Journals

Journal of Animal Science and Veterinary Medicine
Volume 6(1), pages 14-19, February 2020
Article Number: 89791CEE3
ISSN: 2536-7099
https://doi.org/10.31248/JASVM2020.196
https://integrityresjournals.org/journal/JASVM

Full Length Research

Fish entrails meal as feed for broilers *(Gallus gallus domesticus)*: Its potential as dietary supplements on the carcass quality and meat organoleptic evaluation

Janine I. Berdos[1*], Angel Ann A. Aquino[2], Lloyd B. Garcia[1] and Allan Rey S. Angeles[1]

[1]Department of Animal Science, Tarlac Agricultural University, Camiling, Tarlac 2306, Philippines.
[2]New Hope Liuhe, Gerona, Tarlac, Philippines.

*Corresponding author. Email: janine.berdos@yahoo.com; Tel: +639462284305.

Copyright © 2020 Berdos et al. This article remains permanently open access under the terms of the Creative Commons Attribution License 4.0, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Received 17th March, 2020; Accepted 14th April, 2020

ABSTRACT: The main objectives of the study were to examine the nutritional value, carcass quality and meat organoleptic evaluation of broilers supplemented with fish entrails meal. Proximate analysis of fish entrails meal was analysed. Its effects on carcass weight, dressed weight and cuts-up weight were evaluated when used as supplement in the ration of broilers. For the organoleptic evaluation, hedonic scale scorecard was used. A total of 60 respondents evaluated the poultry meat fed with fish entrails meal and each sample were randomly assigned in Completely Randomized Design (CRD) following the four treatments. The treatments were: without fish entrails meal, with 3, 5 and 7% fish entrails meal. Fish entrails meal contained 33.0±0.9% crude protein and 38.4±0.9% crude fat. Carcass quality evaluation revealed that supplementing fish entrails meal significantly influenced the dressed yield ($p<0.05$), carcass yield ($p=0.01$), leg weight ($p<0.01$), and breast weight ($p<0.05$). Furthermore, hedonic scale scorecard revealed that the colour of carcass were paled but statistically ($p<0.05$), 7% inclusion rate of fish entrails meal influenced the carcass colour. In terms of economics, broilers fed with 5% fish entrails meal had the highest income over feed cost (43.36 PhP) since it attained highest marketable weight (1,511.11 g/bird). Generally, fish entrails meal contained high crude protein, high crude fat and moderately low in crude fiber. Fish entrails meal as part of the ration supported satisfactorily the carcass quality and organoleptic quality of cobb broilers. Moreover, feeding fish entrails meal up to 5% increased income over feed cost.

Keywords: Fish entrails meal, meat organoleptic.

INTRODUCTION

Poultry industry has been noted by the Philippine Statistics Authority as a growing venture in the country especially today wherein many Filipino farmers switched from pork to chicken production due to African Swine Fever (ASF) disease outbreak (Galang, 2019) which has been a significant contributor to the country's agriculture sector. As of 2018, a broiler population in first quarter of the year was estimated about 184.34 million wherein Central Luzon had the highest broiler inventory with a total share of 79.89% in the country's total broiler production, followed by Calabarzon and Northern Mindanao. The top three (3) chicken producers in the country accounted for 63.70% of the total chicken production (PSA, 2018).

As the broiler production is growing, the needs in terms of vitamins and minerals for optimum growth of broiler are also considered. However, the cost of synthetic vitamins and minerals which are being used is also increasing. With this, the use of an alternative readily sources such as fish entrails could substitute the vitamins (A, D and B complex) and minerals source as well as boost production (Batal and Dale, 2011 and Fanimo et al., 1996).

Fish entrails through the help of the aquaculture industry could be availed anytime since it is a major source of animal protein and income for smallholders in the Philippines. In 2012, the Philippines ranked among the major fish producing countries in the world with a total

production of 3.1 million tonnes of fish, crustaceans, mollusks and other aquatic animals (FAO, 2020). Meanwhile, in the first quarter of 2019, it posted an increase of 0.9% from its previous year's level of 1.00 million metric tons (PSA, 2019). The utilization of fish wastes in the country is known for processing of fish sauce, human consumption, production of commercial fish meal and direct feed to aquaculture species (Cruz, 2014) but not common for the production of locally fish entrails meal for small hold poultry raisers. Hence, to lessen the expenses and to minimize the use of synthetic sources in poultry feeds, this study was conducted to evaluate the proximate values of fish entrails meal as dietary supplement for broilers and to determine the carcass quality of broilers as influenced by fish entrails meal.

MATERIALS AND METHODS

Animals and treatments

A total of 120 fourteen-day-old Cobb broiler chickens with an average weight of 261.13 g were used as part of feeding trial. The Cobb broiler chickens were randomly distributed to four experimental treatments following Completely Randomized Design (CRD). Each treatment had three replicates with 10 mixed-sex chickens for each replicate. The experimental treatments were: (1) Commercial Ration (CR) only, (2) CR+ 3% fish entrails meal, (3) CR + 5% fish entrails meal, and (4) CR + 7% fish entrails meal.

Fresh mixed-fish entrails from *Chanos chanos*, *Oreochromis niloticus*, *Decapterus macarellus* were purchased in the public market, boiled at 100ºC, and it was heated, roasted and well-cooked in a pan. The dried fish entrails were manually ground using mortar and pestle until homogenous texture was obtained. The fish entrails meals were weighed using a digital weighing scale and mixed with the commercial ration (Table 1) based on the inclusion rate needed.

Data collection

A 200 g sample of dried fish entrails were placed in separate zip lock plastic bags marked and sealed and personally sent to the Department of Agriculture, Regional Field Office III (DA-RFO III)-Regional Feed Chemical Analysis Laboratory, City of San Fernando, Pampanga, Philippines for proximate analysis.

To evaluate the carcass and cut yields, Cobb broiler chickens were weighed individually at day 36 (end of the experiment) following the Animal Welfare Act in the Philippines (Republic Act No. 8485, Section 6, No.6). Three (3) chicken per replication were fasted for six (6) hours and they are only allowed to drink water *ad libitum* prior to slaughtering. Chicken were also weighed before subjected to slaughter (stunning, bleeding, plucking, chilling, and dripping) following the slaughtering procedures implemented by Department of Agriculture (DA)-Administrative Order No.18, Series of 2008. The carcass and cut-ups yields were recorded.

To evaluate organoleptic quality of chicken meat, meat samples with an equal average weight was randomly selected from the cut-ups yield. The raw meat samples were steamed at 80ºC for 15 to 30 minutes until desired texture were obtained and additional of any spices, seasonings or condiments were not necessary. The meat samples were cut to approximately 2.5 inches cubes and were served once from each experimental-group, and the serving order was randomized according to sample, replicate and assessor. Intensities of tenderness, juiciness, flavour, aroma and desirability were evaluated with the use of hedonic scale scorecard. The hedonic scale scorecard (Table 5) used for evaluation of sensory attributes ranging from the lowest intensity of each attributes (score 1) to the highest intensity (score 5). Water were served for cleansing the palate between samples. Sixty panelists composed of 20 faculty members, 20 non-faculty members, 10 male students and 10 female students participated in the organoleptic evaluation which is within the required range.

Statistical analysis

The five-point hedonic scale scorecard were used to determine the organoleptic quality of poultry meat fed with fish entrails meal and data were analyzed using ANOVA of STAR (Statistical Tool for Agricultural Research). For carcass quality, data were analyzed using ANOVA of STAR (Statistical Tool for Agricultural Research). The least-significant differences (LSD) test was used to determine significant differences between treatment means at $p = 0.05$.

RESULTS AND DISCUSSION

Nutritional value

The proximate analysis generally typified the chemical composition of fish entrails meal (Table 2). Results indicated that the crude protein was the main interest of study where it was expected to influence carcass of broilers.

The amount of crude protein (Table 2) was high and consistent to study conducted by Gibson and Hotz (2001) in which fish entrails was an excellent source of animal protein and minerals for poultry. However, crude protein values in recent study was much lower than the chemical composition found by Arvanitoyannis and Kassaveti (2008) due to differences of fish species and processing techniques adapted. This was also supported by Rosenfeld et al. (1997) where they observed that the

Table 1. Nutrient Standards for poultry feeds as basis of commercial producers.

Proximate analysis	Feed Type		
	Chick booster	Broiler starter	Broiler finisher
Crude Protein (%NLT)	21.50	19.50	18.00
Crude Fat (%NLT)	4.00	5.00	6.00
Crude Fiber (%NMT)	3.50	4.50	5.50
Moisture (%NMT)	12.00	12.00	12.00
Calcium (%)	0.85-1.15	0.80-1.10	0.80-1.10
Phosphorus (%NLT)	0.70	0.70	0.70

Source: PhilSAN (Philippine Society of Animal Nutritionists), 2010.

Table 2. Proximate composition (%) of FEM.

Parameter	Results
Dry matter	95.2
Crude protein	33.0 ± 0.9
Ash content	19.0 ± 0.5
Crude fat	38.4 ± 0.9
Crude fiber	1.5 ± 0.5
Nitrogen-free extract	8.1

Table 3. Carcass quality of broiler in terms of slaughter weight (g), dressed and carcass yields (%) as influenced by FEM on their rations.

Treatment	Slaughter weight (g)	Dressed yield (%)	Carcass yield (%)
T1 – Commercial Ration (CR)	1,288.88	89.05^{b}	63.97^{c}
T2- 3% FEM + CR	1,388.88	89.60^{ab}	64.32^{bc}
T3 - 5% FEM+ CR	1,511.11	91.54^{a}	68.60^{ab}
T4 - 7% FEM + CR	1,432.22	96.99^{a}	74.63^{a}
p-value	0.5122	0.0389	0.0136

In each column, means followed by different superscript letters are significantly different ($p<0.05$).

processing method used can directly affect the nutritional value of the meal and proved that in general, amino acid content and protein quality of animal protein sources tend to be superior than those of vegetable sources.

Carcass quality

The carcass quality in terms of slaughter weight was not affected ($p>0.05$) by the inclusion of fish entrails meal (FEM) (Table 3). However, statistics revealed significant effect on supplementing 7% FEM in their ration as compared to non-FEM ration. Thus, numerically, broilers given 7% FEM in the ration were the highest among its counterparts with 96.99% dressed yield ($p<0.05$) and 74.63% carcass yield ($p=0.01$).

The weights of leg and breast in terms of percent yield were affected when FEM was included in their diet (Table 4). The highest percentage ($p<0.01$) of leg yield (37.19%) was seen in broilers supplemented with 5% FEM in their ration while breast yield (33.05%) was consistently observed ($p<0.05$) in broilers supplemented with 7% FEM in their ration.

FEM significantly increased in dressed and carcass yields was associated with the study conducted by Ponce and Gernat (2002) where tilapia by-products could partially replace the use of soybean meal in broiler diets without negatively affecting performance or carcass quality. On the other hand, Salih (2009) claimed that broilers fed fish meal at levels 0, 1.5, 3.5 and 5% improved carcass meat quality. This finding indicated that 7% FEM in the ration of poultry is regarded as satisfactory.

Organoleptic quality

The results of organoleptic quality through sensory evaluation (colour, odor, texture, taste, general acceptability)

Table 4. Cut-ups yield (%) as influenced by FEM on their rations.

Treatments	Leg yield (%)	Breast yield (%)
T1 – Commercial Ration (CR)	34.50^{b}	31.54^{c}
T2- 3% FEM + CR	30.97^{b}	32.34^{bc}
T3 - 5% FEM+ CR	37.19^{a}	32.05^{ab}
T4 - 7% FEM + CR	30.35^{b}	33.58^{a}
p-value	0.0030	0.0165

In each column, means followed by different superscript letters are significantly different ($p<0.05$).

Table 5. Five-point hedonic scale of sensory evaluation.

Score	Organoleptic quality				
	Color	Odor	Texture	Taste	General acceptability
1	Dark	Dislike	Very poor	Very poor	Dislike
2	Slightly dark	Neither like	Poor	Poor	Neither like
3	Moderate	Like slightly	Fair	Fair	Like slightly
4	Pale	Like moderately	Good	Good	Like moderately
5	Very pale	Like very much	Very good	Very good	Like very much

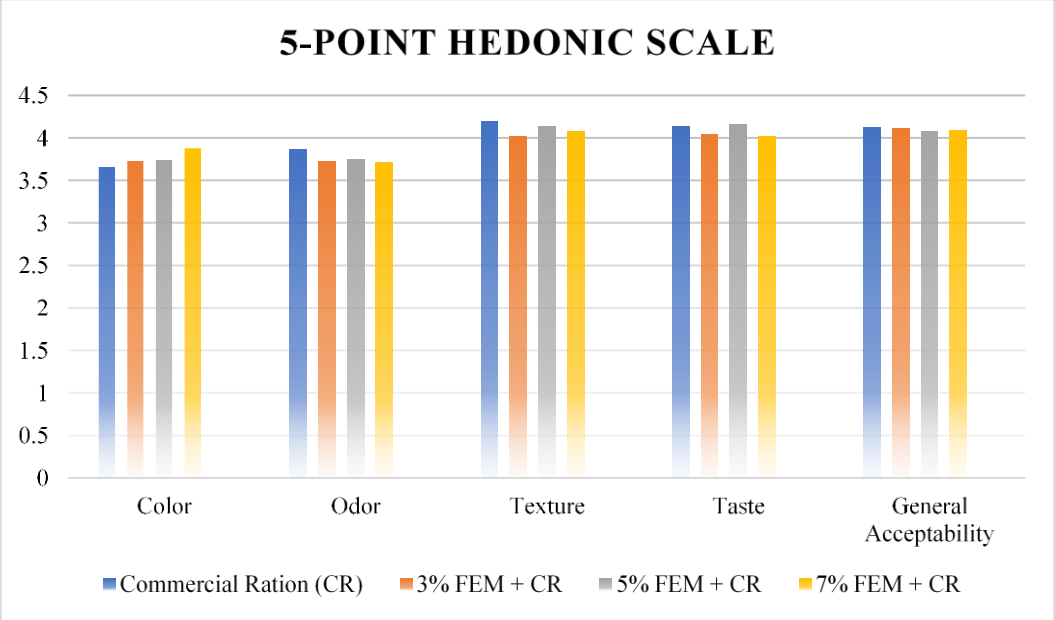


Figure 1. Organoleptic evaluation result of poultry meat as influenced with FEM by their ration based on 5-point scale score card.

was obtained from steamed poultry meat supplemented with (FEM) in their ration. Figure 1 shows the average values obtained based on hedonic scales by respondents (tasters). Although inclusion of FEM in their ration had no significant effect with the colour of steamed meat, which remarked pale, statistically, 7% FEM in their ration has little influenced ($p>0.05$) on its meat colour. Furthermore, taste and texture of the meat were not influenced as panelists classified it as good. Likewise, odor and general acceptability of meat were described as like moderately by panelists (Table 5).

The process of cooking meat is an utmost considered factor in changes of colour that affects the concentration and chemical form of myoglobin, morphology of muscle structure and the ability of the muscle to absorb or scatter incident light which might resulted to pale or dark colour of

Table 6. Income over feed cost of broilers supplemented with and without FEM in their rations.

Item	Commercial ration	3% FEM+CR	5% FEM+CR	7% FEM+CR
Fish entrails[1], Php	-	3.68	6.35	9.27
Marketable weight[2], g	1,288.88	1, 388.88	1,511.11	1,432.22
Income over feed cost[3], PhP	6.63	22.75	43.36	40.00

[1]Price of fish entrails is Php 5.00/kg but may vary depending on place. 2Average marketable weight per broiler for 5 weeks.3Income over feed cost per broiler.

the meat (Walters, 1975 as cited in Seideman et al., 1984). In contrary, Al-Marzooqi et al. (2010) considered the type of a housing as a factor for having yellowish colour of meat for birds raised in open-sided house compared from birds raised in close house while diet can affect its juiciness and flavour. The present study was not influenced by odor, however, 75% inclusion rate of local fish meal in their ration would result to fishy flavour and odor in carcasses (Jassim, 2010). Likewise, birds fed diet of 30% fish silage would give an off-flavour or fishy odor to meat. The present data indicate that 3 to 7% FEM inclusion in their ration does not deteriorate the quality of meat.

Income over feed cost

Overall, the highest income over feed cost (PhP) was obtained in broilers supplemented with 5% FEM in their ration with a value of PhP 43.36/bird and a marketable weight of 1,511.11 g/bird compared with other treatments (Table 6).

The broiler supplemented with and without FEM differed in marketable weight produced. In line with this, supplementation of 3 to 7% FEM is acceptable in terms of their marketable weight and economics of production (Okah and Onwujiariri, 2012; Awoniyi et al., 2003). Aside from that, replacement of costly fish meal with conventional dietary fish meal would increase profitability and meat yield (Hossain et al., 2003). Therefore, inclusion of FEM in their ration is tolerable due to certain areas or countries where large amounts of fish meal are produced that may be economically feasible to use fish meal as the major protein supplement in the diet of broiler chicks (Ponce and Gernat, 2002).

Conclusion

In conclusion, fish entrails meal contained high crude protein, high crude fat and moderately low in crude fiber. Fish entrails meal as part of the ration supported satisfactorily the carcass quality and organoleptic quality of cobb broilers. Moreover, feeding fish entrails meal increased income over feed cost.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENT

We acknowledge the College of Agriculture and Forestry of Tarlac Agricultural University for partly supporting the work in terms of Animal Processing Laboratory.

REFERENCES

Al-Marzooqi, W., Al-Farsi, M. A., Kadim, I. T., Mahgoub, O., & Goddard, J. S. (2010). The effect of feeding different levels of sardine fish silage on broiler performance, meat quality and sensory characteristics under closed and open-sided housing systems. *Asian-Australasian Journal of Animal Sciences*, *23*(12), 1614-1625.

Arvanitoyannis, I. S., & Kassaveti, A. (2008). Fish industry waste: treatments, environmental impacts, current and potential uses. *International Journal of Food Science and Technology*, *43*(4), 726-745.

Awoniyi, T. A. M., Aletor, V. A., & Aina, J. M. (2003). Performance of broiler-chickens fed on maggot meal in place of fishmeal. *International Journal of Poultry Science*, 2(4), 271-274.

Batal, A., & Dale, N. (2011). *Feedstuffs ingredient analysis table (2011 Edition)*. University of Georgia, United States. Pp.16-19.

Cruz, W., Villanueva, J., & Janeo, E. (2015). Status development and use of alternative dietary ingredients in aquaculture feed formulation in the Philippines. In: Catacutan, M. R., Coloso, R. M., & Acosta, B. O. (eds.). Development and use of alternative dietary ingredients or fish meal substitutes in aquaculture feed formulation: *Proceedings of the ASEAN regional technical consultation on development and use of alternative dietary ingredients or fish meal substitutes in aquaculture feed formulation, 9-11 December 2014, Nay Pyi Taw, Myanmar (pp. 23-29).* Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.

Department of Agriculture (DA)-Administrative Order No.18 (2008). Rules and regulations on humane handling in the slaughter of animals for food. Republic of the Philippines, Department of Agriculture Administrative Order No. 18, Series of 2008.

Fanimo, A. O., Mudama, E., Umukoro, T. O., & Oduguwa, O. O. (1996). Substitution of shrimp waste meal for fish meal in broiler chicken rations. *Tropical Agriculture*, 73(3), 201-205.

FAO (2020). Food and agriculture organization, fisheries and aquaculture department, fishery and aquaculture country profiles, the republic of the Philippines. Available: Retrieved 31th March 2020 from http://www.fao.org/fishery/facp/PHL/en.

Galang, V. (2019). Business world. Retrieved 2nd April 2020 from https://www.bworldonline.com/poultry-industry-maintains-2019-growth-target/.

Gibson, R. S., & Hotz, C. (2001). Dietary diversification/modifica-

tion strategies to enhance micronutrient content and bioavailability of diets in developing countries. *British Journal of Nutrition*, *85*(S2), S159-S166.

Hossain, M. H., Ahammad, M. U., & Howlider, M. A. R. (2003). Replacement of fish meal by broiler offal in broiler diet. *International Journal of Poultry Science*, 2(2), 159-163.

Jassim, J. M. (2010). Effect of using local fish meal (Liza abu) as protein concentration in broiler diets. *International Journal of Poultry Science*, 9(12), 1097-1099.

Okah, U., & Onwujiariri, E. B. (2012). Performance of finisher broiler chickens fed maggot meal as a replacement for fish meal. *Journal of Agricultural Technology*, *8*(2), 471-477.

PhilSAn (2010). *Feed Reference Standards (4th edition)*. Philippine Society of Animal Nutritionists, Philippines. p.271.

Ponce, L. E., & Gernat, A. G. (2002). The effect of using different levels of tilapia by-product meal in broiler diets. *Poultry Science*, 81(7), 1045-1049.

PSA (2018). Philippine statistics authority, chicken situation report: January to March 2018. Retrieved 18th May, 2019 from https://psa.gov.ph/content/chicken-situation-report-january-march-2018.

PSA (2019). Philippine statistics authority, fish situation report: January to March 2019. Retrieved 18th March, 2019. https://psa.gov.ph/content/fisheries-situation-report-january-march-2019.

Republic Act 8485 (1998). An act to promote animal welfare in the Philippines, otherwise known as “The Animal Welfare Act of 1998”. Republic Act No. 8485, February 11, 1998.

Salih, G. E. (2009). Effects of inclusion of local disposed roasted fish meal on the performance and carcass characteristics of broilers. *Egyptian Poultry Science Journal*, 29(3), 735-745.

Seideman, S. C., Cross, H. R., Smith, G. C., & Durland, P. R. (1984). Factors associated with fresh meat color: a review. *Journal of Food Quality*, 6(3), 211-237.

International Journal of Research and Review
DOI: https://doi.org/10.52403/ijrr.20210539
Vol.8; Issue: 5; May 2021
Website: www.ijrrjournal.com
Review Paper *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

An Overview of Smart Farming Production Technology for the Advancement of Home-grown Farmers in the Philippines

Jeffrey John R. Yasay

Department of Computer Studies, College of Engineering and Technology, Tarlac Agricultural University, Camiling Tarlac, Philippines.

ABSTRACT

This article explores the technologies that can be used to establish smart farming in the Philippines, as well as the various smart systems that have been used to aid home-grown farmers. The emergence of smart agriculture and farming is a method that heavily integrates digital technology in order to increase food production while minimizing input costs. The importance of this technology has a significant effect on farmers and investors as a result of technological advancements. It should also be recognized that numerous promotions requiring government funding for the establishment of smart farming technology in the Philippines has been addressed.

Keywords: Smart Farming; Hydroponics; Aquaponics; Aeroponics

INTRODUCTION

In the Philippines, almost half of the population lives in rural areas and relies on agriculture for a living; among them are indigenous people, landless farmers, and fishermen [1]. In general, farmers on different islands in the Philippines operate independently using conventional methods, and their management of farm produce to end-users is facilitated at low prices by middlemen. Micro-propagation protocols for bananas, coconuts, legumes, and oilseed crops are well known [2].

In the first quarter of 2021, the value of agricultural output fell by -3.3 percent at constant 2018 rates. This was attributed to a decrease in livestock and poultry demand. Crops and fisheries, on the other hand, also increased productivity [3]. Despite this condition, the Philippines is working to modernize and improve its agriculture industry, with both the government and private firms encouraging the use of advanced technologies and smart farming practices to raise harvests and reduce losses [4].

Agriculture's creation was a watershed moment in human history. The willingness of fully modern humans to change the atmosphere to produce enough food to support population growth is the first major improvement in the relationship between fully modern individuals and society. Agriculture ushered in a slew of new developments, ranging from the use of fire and cooked food to self-driving machinery [5].

Hence, smart farming is seen as the agricultural future because it produces higher quality crops by making farms more intelligent in sensing their controlling parameters [6].

SIGNIFICANCE OF SMART FARMING TECHNOLOGY

Agriculture routinely uses sophisticated technologies such as robots, temperature and moisture sensors, aerial images, and GPS technology. These cutting-edge devices, precision agriculture, and robotic systems enable businesses to be more profitable, efficient, safe, and environmentally friendly [7].

Thus, technology is critical to the development of the farming industry and the improvement of agribusiness. Researchers have successfully grown crops in deserts and other harsh environments using genetic engineering, which involves inserting traits into established genes in order to produce pest-resistant, drought-resistant, and plant pathogen-resistant crops.

Moreover, this technology will enhance insect or pest resistance, herbicide or drought tolerance, and disease resistance, providing farmers with a new tool for increasing crop yield. Farmers have used plant breeding and selection techniques to increase crop yield with the assistance of researchers. Technology is also used to protect crops by tracking growth and detecting plant diseases. Without the physical involvement of farmers, automation allows for the consistent distribution of fertilizers, pesticides, and water throughout fields [8].

Lastly, innovative agriculture ensures that new farming and agricultural development models emerge, introducing innovative techniques on how food is produced and distributed. These methods allow more economies and regions to keep up with changing trends and meet the demands of modern living while ensuring sustainably grown food. [9].

SMART FARMING TECHNOLOGY

Hydroponics Farming

Hydroponic farming is a method of growing plants in water without soil using mineral nutrient solutions. The hydroponic gardener controls the nutrient content of the liquid solution used to water the plants [10].

Common Types of Hydroponics System

1. Nutrient Film Technique (NFT)

A method of cultivating plants in which plant roots grow in shallow and circulating hydroponic nutrient layers, allowing plants to receive adequate water, nutrients, and oxygen. Plants grow in layers of polyethylene, with plant roots immersed in nutrient-rich water that is constantly pumped by a pump [11].

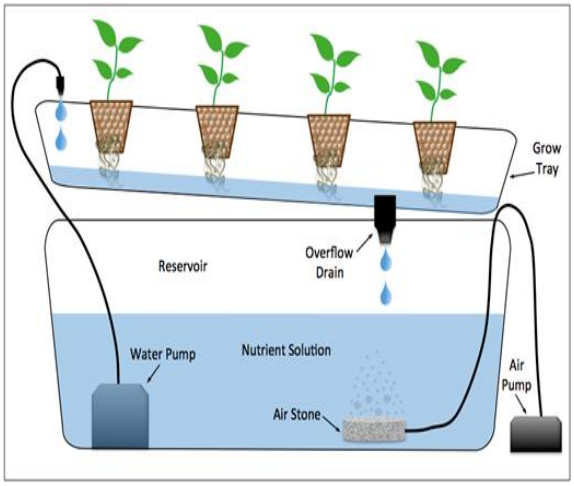


Figure 1. Diagram of the Nutrient Film Technique (NFT) hydroponic system [12]

2. Wick Systems

It is considered the most basic hydroponic device. The Wick system is classified as a passive system, which means it has no moving parts. Your unique Growth Technology nutrient solution is drawn up into the expanding medium through a number of wicks from the bottom reservoir. This device will work with a number of mediums, including perlite, soil, and coco [13].

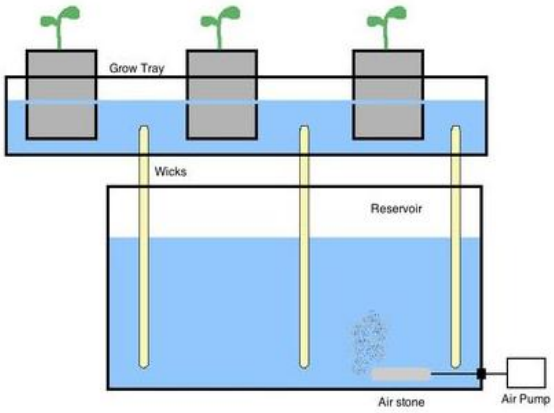


Figure 2. Diagram of the Wick System [14]

3. Deep Water Culture (DWC)

It is a hydroponic method of plant production by suspending the roots of the plant in a solution of oxygenated, rich in nutrients. This system uses rectangular tanks of less than one foot deep filled with a nutrient-rich solution and plants floating on

Styrofoam panels, also known as Deep Flow Technique (DFT), Floating Raft Technology (FRT), or Raceway [15].

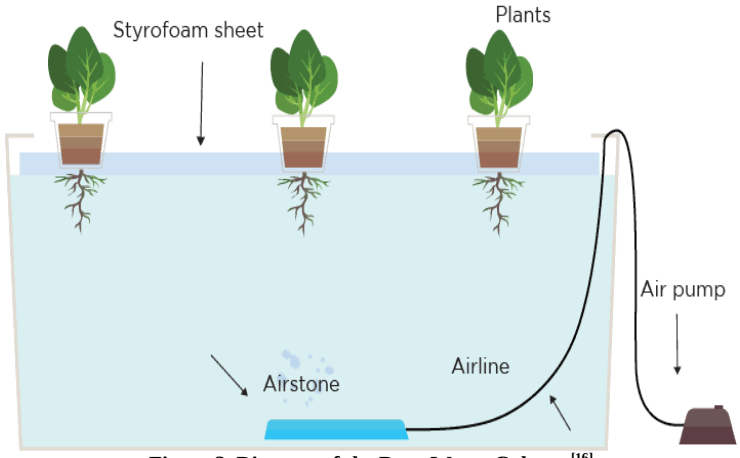


Figure 3. Diagram of the Deep Water Culture [16]

4. Ebb and Flow (Flood and Drain)

It is a hydroponics technique that involves flooding the growth media with nutrient solution for a set period of time, after which the unabsorbed nutrient is returned to the tank. Normally, this hydroponics device uses a timer to fill the water, resulting in inefficient usage of nutrient solution [17].

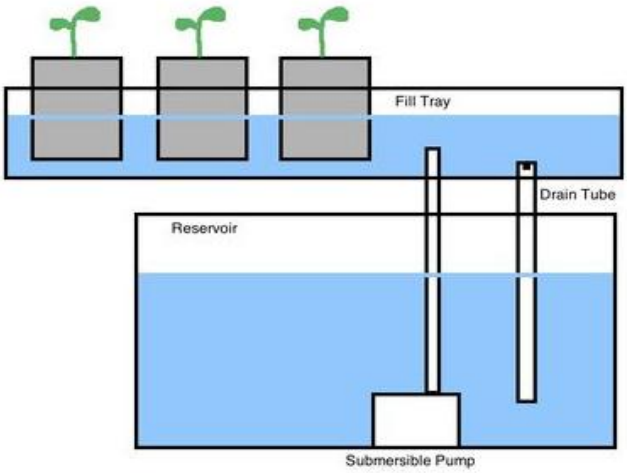


Figure 4. Diagram of the Ebb and Flow [18]

Aquaponics Farming

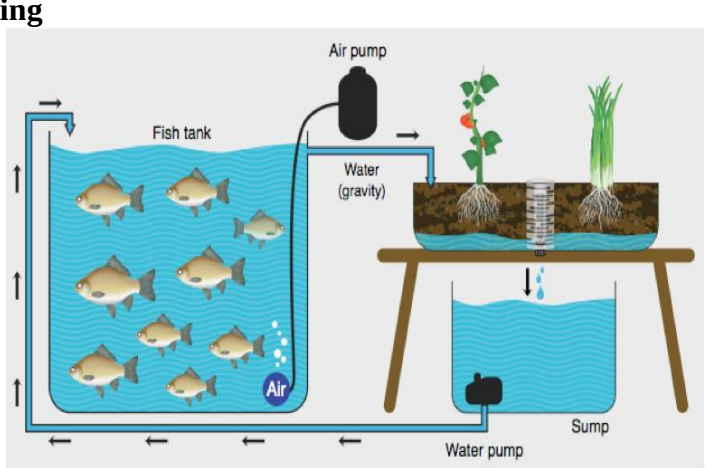


Figure. 5. Diagram of the Aquaponics [19]

In an aquaponics system, water from an aquaculture system is fed into a hydroponic system where by-products' are broken down by nitrifying bacteria first into nitrites and then into nitrates, which are used as nutrients by the plants [19]. A symbiotic relationship between two food production disciplines: (1) aquaculture, the farming of aquatic species, and (2) hydroponics, the cultivation of plants in water without soil. Aquaponics is a closed recirculating device that incorporates the two. A typical recirculating aquaculture system filters and eliminates organic matter ("waste") that accumulates in the water, ensuring that the water is safe for the fish [20].

Aeroponics Farming

In Aeroponics, the nutrient solution is sprayed onto the roots by moving it through misters inside the root region, either continuously or several times per hour [21].

The plant you want to develop is suspended in an air space with an atmosphere that is either completely closed or semi-closed. As a result, it is best achieved in a closed, regulated environment where you can monitor the amount of light, air, and nutrient-rich water spray that is fed into the plant [22].

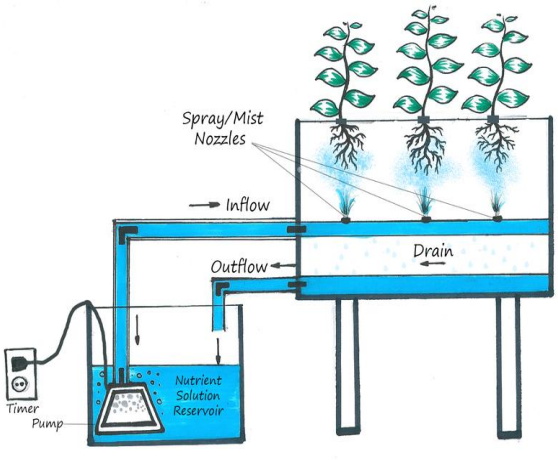


Figure 6. Diagram of Aeroponics [22]

GOVERNMENT SUPPORT TO SMART FARMING

In the Philippines, the local government, led by the Department of Agriculture, is aiming for a 2.5 percent growth this year through further incorporation of technology in agriculture to increase productivity, connectivity, and service delivery to beneficiaries. By focusing on and closely implementing 'Agriculture 4.0,' or the fourth agricultural revolution that encourages the use of smart farming technology, the country would have a better chance of having a better 2021 in terms of agriculture [23].

Agriculture Secretary William Dar released a memorandum to all DA executives, attached agencies and companies, services, and regional offices directing them to "pursue an inclusive approach on these main strategies to accelerate the transition into a new and industrialized Philippine agriculture." [24].

Another agency distinguished in its Labor Market Intelligence report "Soils to Satellites," the Technical Education and Skills Development Authority (TESDA) has been published covering practical topics such as automation in smart greenhouses, agricultural drones, IoT solutions to agricultural problems, and case studies in selected ASEAN countries in smart agriculture applications [25].

CONCLUSION

Some technologies will need to be developed specifically for agriculture, while other technologies already developed for other areas could be adapted to the modern agricultural domain such as autonomous vehicles, artificial intelligence and machine vision and smart farming.

Moreover, as farming in the Philippines faces several problems, proactive solutions like ICT must be implemented together with the full support of the government. Similarly, other major players, such as multinational companies, agricultural and fisheries industry leaders and organizations, and agricultural state

universities and colleges (SUCs), should work together to elevate home-grown farmers in the country.

Lastly, if modern agriculture is applied widely in the near future, millions of farmers will be able to benefit from the acquisition and development of smart farming production technology.

ACKNOWLEDGEMENT

This study is not possible without the help of the Tarlac Agricultural University.

Conflict of Interest: None

Source of Funding: None

REFERENCES

1. Briones, Z.B.H., Yusay, R.M.S. and Valdez, S. (2017), "Enhancing community based tourism programs of Gawad Kalinga enchanted farm towards sustainable tourism development", Journal of Economic Development, Management, IT, Finance, and Marketing, Vol. 9 No. 1, pp. 51-60.
2. H.P. Singh, S. Uma, R. Selvarajan and J.L. Karihaloo, "Micropropagation for production of quality banana planting material in Asia-Pacific," Asia-Pacific Consortium on Agricultural Biotechnology (APCoAB), New Delhi, India, vol. 92, 2011.
3. Mapa, D. S. (2021, May 10). Agricultural production dropped by -3.3 percent in the first quarter of 2021. Retrieved May 20, 2021, from https://psa.gov.ph/content/agricultural-production-dropped-33-percent-first-quarter-2021
4. Oxford Business Group. (2019, April 03). New smart farm to help the Philippines achieve sustainable Agriculture goals. Retrieved January 8, 2021 from https://oxfordbusinessgroup.com/news/new-smart-farm-help-philippines-achieve-sustainable-agriculture-goals
5. Ku, L.(2019, May 21). How Automation is Transforming the Farming Industry. Retrieved February 10, 2021 from https://www.plugandplaytechcenter.com/resources/how-automation-transforming-farming-industry/
6. Alipio, M. I., Dela Cruz, A. E. M., Doria, J. D. A., & Fruto, R. M. S. (2019). On the design of Nutrient Film Technique hydroponics farm for smart agriculture. Engineering in Agriculture, Environment and Food. doi:10.1016/j.eaef.2019.02.008
7. National Institute of Food and Agriculture. Agriculture Technology | National Institute of Food and Agriculture. (n.d.). https://nifa.usda.gov/topic/agriculture-technology.
8. Media, J. L. I. S. (2019, February 22). Importance of Modern Technology in Agribusiness: JLI Blog. JLI Blog | Global Training & Education Provider. https://www.jliedu.com/blog/modern-technology-agribusiness/
9. ThistlePraxis. (2018, August 10). Innovations in agriculture: Ideas, Possibilities, Strategies (II). https://medium.com/@ThistlePraxis/innovations-in-agriculture-ideas-possibilities-strategies-ii-86942087847a.
10. Cosgrove, C. (2020, May 9). Introduction to Hydroponic Farming. Blogging Hub. https://www.cleantechloops.com/hydroponic-farming/.
11. Iswanto, P. Megantoro and A. Ma'arif, "Nutrient Film Technique for Automatic Hydroponic System Based on Arduino," 2020 2nd International Conference on Industrial Electrical and Electronics (ICIEE), 2020, pp. 84-86, doi: 10.1109/ICIEE49813.2020.9276920.
12. Diagram of the Nutrient Film Technique (NFT) hydroponic system: Hydroponics system, Hydroponics, Aquaponics system. Pinterest. (2018). https://www.pinterest.ph/pin/548876891871 36121/.
13. Shailesh Solanki1, Nitish Gaurav, Geetha Bhawani and Abhinav Kumar. (2017); Challenges And Possibilities In Hydroponics: An Indian Perspective. Int. J. of Adv. Res. 5 (Nov). 177-182]
14. D'Anna, C. (2019, October 3). How to Use the Wick System Method in Your Hydroponic Garden. The Spruce. https://www.thespruce.com/hydroponic-gardens-wick-system-1939222.
15. Roberts, Olu (August 2019). "Food safety handbook for hydroponic lettuce production in a deep water culture"
16. Max. (2021, February 1). Deep Water Culture (DWC) - The Definitive Guide.

Trees.com. https://www.trees.com/gardening-and-landscaping/deep-water-culture.
17. Daud, Muhammad & Handika, Vandi & Bintoro, Andik. (2018). Design And Realization Of Fuzzy Logic Control For Ebb And Flow Hydroponic System. International Journal of Scientific & Technology Research. 7. 138-144.
18. D'Anna, C. (2019, July 22). Your Guide to Ebb and Flow Systems of Hydroponic Gardens. The Spruce. https://www.thespruce.com/hydroponic-gardens-ebb-and-flow-systems-1939219.
19. Angela. (2019, August 28). Aquaponic System Israel. https://aquaponictrend.blogspot.com/2018/01/aquaponic-system-israel.html.
20. Campanhola, C., & Pandey, S. (2019). Sustainable food and agriculture: an integrated approach. Academic Press, is an imprint of Elsevier.
21. Mattson, N., & Lieth, J. H. (2019). Liquid Culture Hydroponic System Operation. Soilless Culture, 567–585. doi:10.1016/b978-0-444-63696-6.00012-8
22. Wise, K. (2020, August 26). What Is Aeroponics Farming & Why You Should Care? Medium. https://medium.com/krishi-wise/what-is-aeroponics-farming-why-you-should-care-238617517711.
23. Smart Agriculture and Farming in Philippines 2021 Industry Updates. Companies and products from Asia. (2021, January 26). https://asianavigator.com/smart-agriculture-and-farming-in-philippines-2021-industry-updates,n62.html.
24. Da. (2021, January 5). DA lines up key strategies to steer agri-fishery growth, transformation in 2021. PIA News. https://pia.gov.ph/news/articles/1063102.
25. Maghirang, T. (2020, September 3). AGRI-TECH: Agriculture 4.0 : Eight essential things you need to know. TECHSABADO. https://techsabado.com/2020/09/03/agriculture-4-0-eight-essential-things-you-need-to-know/.

How to cite this article: Jeffrey John R. Yasay. An overview of smart farming production technology for the advancement of home-grown farmers in the Philippines. *International Journal of Research and Review*. 2021; 8(5): 310-315. DOI: *https://doi.org/10.52403/ijrr.20210539*

******

Yancy Ogdamin Waminal et al./Animal Production. 23(3): 144-150, 2021
Accredited by Kemenristek Dikti No 32a/E/KPT/2017. ISSN 1411-2027

Mastitis Detection in Holstein Sahiwal Crossbred Cattle (*Bos taurus*) Using Different Brands and Dilution Levels of Liquid Anionic Surfactants

Yancy Ogdamin Waminal[2]*, Marjorie Raboy Gambol[1], Ahron Caiman Sabado[1], John Paul Muring Vallejos[1], Ronaldo Curameng Briones[2] and Esteward Jones Manlangit Estabillo[2]

[1]Undergraduate Student, Department of Animal Science, College of Agriculture and Forestry, Tarlac Agricultural University, Tarlac, Central Luzon, Philippines
[2]Department of Animal Science, College of Agriculture and Forestry, Tarlac Agricultural University, Tarlac, Central Luzon, Philippines
***Corresponding author email: waminal0830@gmail.com**

Abstract. The study was conducted to evaluate the effect, consistency, and economic viability of using different brands and dilution levels of liquid anionic surfactants to detect Mastitis in dairy cattle. Fifty-five milk samples were collected and subjected to macroscopic evaluation at a cattle farm located in Sta. Maria, Bulacan, Philippines. Milk samples were brought to the Philippine Carabao Center National Headquarters and Genepool Science City of Muñoz, Nueva Ecija for Somatic Cell Count. The experiment used 10 treatments with 3 replications arranged in a Completely Randomized Design. The macroscopic evaluation results showed that liquid anionic surfactants at 30%-50% dilution levels could detect Mastitis in dairy cattle. This was validated by the somatic cell count results from PCC Dairy Laboratory. On the other hand, statistical analysis results revealed that the treatments and the control (commercial mastitis test) were comparable except for brand Z at a 40% dilution rate. Brand Z at 30% and 50% dilution rate were found to be consistent among other treatments in detecting Mastitis in dairy cattle. In terms of economic viability, brand X and brand Y at 30% were the cheapest and had the lowest production cost and highest percentage savings.

Keywords: liquid anionic surfactant, mastitis, milk sample, dairy cattle, somatic cell count

Introduction

Milk is considered a complete food being secreted by female mammals to nourish their young. Several problems can occur in this gland, and the most common is mastitis. Mastitis is a common disease in mammals including dairy livestock industry, which is very detrimental since it decreases the quantity and quality of milk production (Syamsi et al., 2019).

Mastitis is a prevalent disease that affect the mammary gland causing inflammation that is painful to dairy animals and reduces milk quality. Poor management practices or environmental micro-organisms cause the disease. The dairy industry paid more attention to subclinical and clinical Mastitis because of its negative effect on the animal and dairy farms' economic status worldwide. Through early detection of Mastitis, treatments can be employed immediately to reduce the infection and the transmission to other cows.

Although commercial mastitis tests are available, their use in the Philippines are limited. These test kits are expensive and not accessible to some dairy farmers. Moreover, local farmers are not trained of conducting mastitis test for milk. It is necessary to find a material that has the same effect but is able to accommodate the ease of application in farmers.

Given that liquid anionic surfactant does not require expensive solutions, instruments, and technical laboratory skills, it is also accessible; hence, it is efficient to detect mastitis infection in the shortest possible time. Despite improvements made in animal production and health, particularly in prevention by early detection of contagious disease and therapeutic medicine, this study was conducted to determine the efficacy of liquid anionic surfactant as a reagent for mastitis test.

Materials and Methods

Scope and Delimitation of the Study

This study was conducted from January to May 2020. Milk samples were collected at a dairy cattle farm located in Sta. Maria, Bulacan. Milk samples were brought to the Philippine Carabao Center National Genepool Headquarters, Science City of Muñoz, Nueva Ecija for Somatic Cell Count. The study was limited to mastitis detection in dairy cattle using different brands and dilution levels of liquid anionic surfactants.

Experimental Design

A total of 55 milk samples from dairy cattle were used in this study. The experiment was laid out following the Completely Randomized Design (CRD). Treatment with a corresponding dilution level was replicated three (3) times. The 3-day sample collection represented the three (3) replications.

There were ten treatments used in the study as shown in Table 1.

Table 1. Brands and dilution levels of liquid anionic surfactants used in the study

Treatments	Brands and dilution levels of liquid anionic surfactants
1	commercial mastitis test (Control)
2	30% Brand X + 70% Distilled water
3	40% Brand X + 60% Distilled water
4	50% Brand X + 50% Distilled water
5	30% Brand Y + 70% Distilled water
6	40% Brand Y + 60% Distilled water
7	50% Brand Y + 50% Distilled water
8	30% Brand Z + 70% Distilled water
9	40% Brand Z + 60% Distilled water
10	50% Brand Z + 50% Distilled water

Preparation of the Test Solution

All the necessary equipment used were thoroughly cleaned, disinfected, and air-dried to prevent contamination of milk samples.

Commercial Mastitis Test and Liquid Anionic Surfactant Test Solution Preparation

On the preparation of the Commercial Mastitis Test Solution, the diluting distilled water was first tested by using 1-2 drops of reagent concentrate. The second step was mixing 10% of reagent and 90% water and was stirred gently in a beaker to avoid bubbles. Lastly, the mixture was transferred in the empty bottles and appropriately labeled.

Liquid Anionic Surfactant Test Solution preparation was similar to that of the commercial solution. To make a 500 ml test solution, different percentage of brands of liquid anionic surfactants and distilled water was computed to the desired volume of the test solution. The distilled water's computed amount volume was measured for the different dilution levels using a beaker, then measured the computed amount volume of liquid anionic surfactant and added into the prepared water solution. The mixture was stirred gently to avoid the excessive formation of bubbles. Lastly, the solution was carefully transferred into clean empty container bottles and labeled. The same procedure was employed following the treatments specified in the experiment.

Mastitis Testing Using Different Test Solutions

Mastitis testing on commercial reagent and Liquid Anionic Surfactant had the same procedure. It was done strictly using the following procedures. First, raw milk was collected and placed in a bottle. Five ml of milk from each quarter was placed into the commercial reagent paddle with four compartments labeled as A, B, C, and D. Second, an equal amount of commercial reagent and LAS solution with different brands and dilution levels was added to each quarter in the paddle. Then, the paddle was rotated in a circular motion to mix the milk and the solution thoroughly. Visible reaction disintegrated after about 20 seconds. The reaction was scored visually according to the gel formation: the more gel formation, the higher the score. Results were recorded accurately per animal and per teat.

Collection of Milk Samples for Somatic Cell Count

Before milk collection, 60 pieces of the centrifuge tube, permanent marker, ball pen, record book, and ice chest were prepared. The procedure of collection was done following the protocol of the dairy laboratory. Strictly 50 ml of milk was drawn from four quarters of the udder into the centrifuge tube. The tubes were labeled with the cow number and teat location and immediately store in the ice chest. The cow number, calving date, milk yield per day, and the result of the commercial reagent test for each quarter were recorded in the sheet provided by the Philippine Carabao Center dairy laboratory.

Laboratory Analysis

Milk samples were collected from the four quarters of the udder from each experimental animal and subjected to macroscopic evaluation.

Table 2. Guide to macroscopic evaluation

No	Result Symbol	Meaning	Description	Remarks
1	-	Negative	Free from the gelatinous formation	Negative of Mastitis
2	-+	Trace	Slight thickening of the mixture. Trace reaction seems to disappear with a continuous rotation of the paddle. If all four quarters read trace, there is no infection. If one or two quarters read trace, infections are possible.	Possible Mastitis Infection
3	+	Weak Positive	A distinct precipitate formed, and milk become slightly viscous, but no tendency of gel formation	Sub-clinical Infection (Grade 1 Mastitis)
4	++	Distinct Positive	The mixture thickens immediately with some gelatinous formation formed.	Serious Mastitis Infection (Grade 2 Mastitis)
5	+++	Strong Positive	A gelatinous formation will be formed and causes the mixture's surface to become convex, and a thick mass is formed in the middle of the paddle while doing swirling and titling. When the mixture is poured out, the whole mass formation will drop without leaving any remains or if there is a presence of little sticky mixture will be left but will drop also	Serious Mastitis Infection (Grade 3 Mastitis)

Note: Formation of gel involves the binding of leukocytes due to the anionic of the liquid antibacterial detergent on the milk. Gelatinous formation usually indicates the quarter of the cow's udder is positive or infected with Mastitis. Source: McCurnin's Clinical Textbook for Veterinary Technicians

Table 3. Somatic cell range and interpretation

Test result Cmt code	Equivalent somatic cell range (cells/ml)	Interpretation
(-) Negative	0-200,000	Healthy Udder
(-+) Trace	> 200,000 – 400 , 000	Sub-clinical Infection. If all four quarters read trace, there is no infection. If one or two quarters read trace, infections are possible.
(+) Grade 1 weak positive	400,000 – 1,200,000	Sub-clinical Infection
(++) Grade 2 distinct positive	1,200,000 -5,000,000	Serious Mastitis Infection
(+++) Grade 3 strong positive	Over 5,000,000	Serious Mastitis Infection

Source: Philippine Carabao Center, National Gene Pool Headquarters – Dairy Laboratory, Science City of Muñoz, Nueva Ecija.

Milk samples were graded the guides in Table 2. Milk samples were also submitted immediately to the Philippine Carabao Center National Gene Pool Headquarters – Dairy Laboratory, Science City of Muñoz, Nueva Ecija, Philippines for Somatic Cell Count Analysis using Cytometry. Based on their recommendations, milk samples were declared infected with mastitis or not using Table 3 as guide.

Data Gathered

The following data were gathered:

1. ***Macroscopic Evaluation Results*** are tabulations in Mastitis Detection using the Commercial reagent and different brands of liquid anionic surfactants.
2. ***Somatic Cell Count Results*** are results of the milk samples collected from the different quarters of the udder of experimental animals that was conducted by PCC.
3. ***Cost of Producing Liquid Anionic Surfactant Agent*** is the computed expenses of producing test solutions.

Data Analysis

All the data gathered were statistically analyzed using the Analysis of Variance (ANOVA) for Completely Randomized Design (CRD). The mean of the results was compared employing the Least Significant Difference test at a 0.01 level of confidence.

Results and Discussion

Mastitis Detection by Macroscopic Evaluation

Results of the macroscopic evaluation for milk samples using commercial mastitis test and different Brands of Liquid Anionic Surfactants (LAS) at different dilution rates are presented in Table 4.

Table 4 shows that Brands X, Y, and Z at 30%-50% dilution rate were able to detect different levels of positive Mastitis like CMT. Brand Z at 30% and 50% dilution level has the highest ability to detect mastitis at its highest grade, i.e., grade 2.

The ability of the LAS to detect mastitis was due to its anionic surface-acting agent akylanylsulfonate – an active ingredient found in surfactants that have the same action in the deoxyribonucleic acid (DNA) reagent which dissolves or disrupts the outer cell wall and the nuclear cell wall of any leukocyte, which are primarily fat. DNA is released from the nuclei. DNA will get together to form a stringy mass. As the number of leukocytes increases, the amount of gel formation also increases linearly.

This is parallel to the result of the study conducted by Gangan et al. (2018) wherein liquid anionic surfactants can be used to detect clinical and subclinical mastitis in dairy animals.

Effectiveness of Liquid Anionic Surfactants at Different Dilution Levels

Presented in Table 5 is the comparison among the different treatments which includes the different brands and dilution levels of liquid anionic surfactants.

Analysis of variance revealed that all treatments have no significant difference to the commercial mastitis test except for treatment 9 (Brand Z at a 40% dilution rate).

This is parallel to the result of the study by Gangan *et al.* (2018) wherein liquid anionic surfactants produced comparable result to commercially available mastitis test reagents.

Consistency of Using Liquid Anionic Surfactants in Mastitis Detection

Results on macroscopic evaluation reveal that the different liquid anionic surfactants and dilution levels were consistently able to detect mastitis dairy cattle's fresh milk. Statistical analysis of variance in Table 4 also reveals the consistency of the liquid anionic surfactants at 30%-50% dilution rate to commercial mastitis test except treatment 9 (brand Z at 40% dilution rate).

Table 4. Results of the macroscopic evaluation using CMT and different brands and dilution levels of liquid anionic surfactants

Treatments	Mastitis grade				
	Negative	Trace	Grade 1	Grade 2	Grade 3
Treatment 1	35	15	5	0	0
Treatment 2	33	18	4	0	0
Treatment 3	31	19	5	0	0
Treatment 4	30	20	5	0	0
Treatment 5	29	21	5	0	0
Treatment 6	31	19	5	0	0
Treatment 7	29	21	5	0	0
Treatment 8	28	23	3	1	0
Treatment 9	18	35	2	0	0
Treatment 10	28	22	4	1	0

Table 5. Mean grade for mastitis detection using the CMT 5-point hedonic scale

Treatments	Treatment id	Means
1	Commercial mastitis test	1.46[a]
2	Brand x at 30 % dilution rate	1.40[a]
3	Brand x at 40 % dilution rate	1.46[a]
4	Brand x at 50 % dilution rate	1.49[a]
5	Brand y at 30 % dilution rate	1.48[a]
6	Brand y at 40 % dilution rate	1.48[a]
7	Brand y at a 50 % dilution rate	1.50[a]
8	Brand z at a 30 % dilution rate	1.50[a]
9	Brand z at a 40 % dilution rate	1.68[b]
10	Brand z at a 50 % dilution rate	1.50[a]

Note: Means followed by the same letter are not significantly different at a 1% level of significance. P(>F) = 0.0005. CV (%) = 3.39

Table 6. Cost efficiency of using different brands of liquid anionic surfactants (LAS) at different dilution levels

Brand and Dilution rate	Cost per gallon (₱)	Cost of CMT/LAS Per 5ml (₱)	Savings from the use of LAS (₱)	Percentage of savings from the use of LAS (%)
Commercial reagent	1,136	1.42		
Brand X at 30%	268	0.335	1.085	323.88
Brand X at 40%	344	0.43	0.99	230.23
Brand X at 50%	420	0.525	0.895	170.48
Brand Y at 30%	268	0.335	1.085	323.88
Brand Y at 40%	344	0.43	0.99	230.23
Brand Y at 50%	420	0.525	0.895	170.48
Brand Z at 30%	304	0.38	1.04	273.68
Brand Z at 40%	392	0.49	0.93	189.80
Brand Z at 50%	480	0.6	0.82	136.67

Therefore, all the treatments were consistent in mastitis detection but treatments 8 and 10 (Brand Z at 30% and 50% dilution rate) were found to be the most consistent among all treatments. This was confirmed by the Somatic Cell Count result (Appendix B) and antibacterial component of the brand can kill bacteria. This result confirms De Guzman *et al*. (2019) that 30% and 50% of brand Z were also consistent in detecting mastitis.

Economic Viability

Presented in Table 6 is the economic viability of using different brands and dilution level of liquid anionic surfactants in detecting subclinical and clinical mastitis in dairy cattle.

Table 6 showed that brand X and brand Y at 30% dilution level obtained the lowest cost per gallon (₱ 268) and cost per sample (₱ 0.335) among all treatments including commercial reagent. In terms of the amount saved using LAS and percentage savings from using commercial reagent, brand X and brand Y at 30% dilution level acquired the highest savings and percentage savings among all treatments with ₱ 1.085 and 323.88%.

Similarly, Dela Cruz *et al.* (2019) stated that using liquid anionic surfactants in detecting Mastitis ruminants was viable because of its availability in the nationwide market and has cheaper cost compared to commercial reagent

Conclusions

Based on the results of the study, the researchers concluded that Brand Z at 30% and 50% dilution levels is the most consistent surfactant solution in detecting mastitis on fresh dairy cattle milk, while Brands X and Y at 30% dilution level are the cheapest solutions to use.

References

Andersen, HJ, LH Petersen, FM Aarestrup, and M Chriel. 2003. Evaluation of the surveillance program of Streptococcus agalactiae in Danish dairy herds. Journal of Dairy Science. 86 (4): 1233-1239

Andersen, S, IR Dohoo, RO Riekerink, and H Stryhn. 2011. Diagnosing intramammary infections: evaluation of definitions based on a single milk sample. Journal of Dairy Science. 94:250-291.

Breen, JE, AJ Bradley, and MJ Green. 2009. Quarter and cow risk factor associated with somatic cell count greater than per milliliter in United Kingdom dairy cows. Journal of Dairy Science. 92 (7): 3106-3115.

Bytyqi, H, U Zaugg, K Sherifi, A Hamidi, M Gjonbalaj, S Muji, and H Mehmeti. 2011. Influence of management and physiological factors on somatic cell count in raw milk in Kosova. Veterinarski Archiv, 80(2):173-183.

Cervinkowa, D, H Vlokova, I Borodacouva, and J Makouvoca. 2013. Prevalence of mastitis pathogens in milk from clinically healthy cows. Veterinary Medicine Journal. 58 (11):567-575.

Cunha, RP, LR Molina, AM Carvalho, and RM Ferreira. 2008. Sub-clinical mastitis and relationship between somatic cell count with a number of lactations, production, and chemical composition of milk. Vet Med. 60:19-24.

Dingwell, RT, KE Leslie, YH Schukken, JM Sargeant, and LL Timms. 2003. Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation dairy cows. Canadian Veterinary Journal. 44: 413.

Dobranie, V, B Njari, B Miokovic, and R Resanova. 2008. The influence of the season on chemical composition and somatic cell count of bulk tank cow's milk. Vet Archive. 78.235-242

El-Attar, AA, ME Salama, and MM Abd El-Samie. 2002. Incidence of mastitis in lactating cows and buffaloes kept under different management conditions in Ismailia province. Veterinary Medicine Journal. 2: 583.

Gangan, JA. 2018. Detection of Mastitis in dairy buffalo using different brands and dilution levels of liquid anionic surfactants.

Halasa, T, and O Osterus. 2007. Economic effects of bovine mastitis and mastitis management. Vet Q. 29:18-31.

Kandasamy, S, BB Green, AL Benjamin, and DE Kerr. 2011. Cow variation in dermal fibrilast response to lipopolysaccharide reflected in resolution of inflammation during E. coli mastitis. Journal of Dairy Science. (94(12):5963-75 doi: 103168 / jds 20111-4288.

Kuang, Y, H Jia, K Miyanaga, and Y Tanji. 2009. Effect of milk on antibacterial activity of tetracycline against Escherichia coli and Staphylococcus aureus isolated from bovine. Applied microbiology and biotechnology. 84. 135-42.

Leslie, KE, RT Dingwell, and LL Tiims. 2002. Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation dairy cows. The Canadian Veterinary Journal. 44(5): 413-416.

Le Roux, Y, and F Moussaqui. 2003. Polymorphonuclear protolyse acting and milk composition change. Vet res. 629-645.

Ma, Y, C Ryan, DM Barbano, DM Gakton, MA Rudan, and KJ Boor. 2000. Effect of somatic cell count on quality and shelf-life of pasteurized fluid milk. Journal of Dairy Science. 83:264-274.

Mahmoud, AA. 1988. Some studies on subclinical mastitis dairy cattle. Assiut Veterinary Medicine Journal. 20: 150.

Ogola, H, and A Shitandi. 2007. Effect of mastitis on raw milk composition quality. Journal of Dairy Science. 8:237-242.

Rady, AA, and M Sayed. 2009. Epidemiological studies on subclinical mastitis in dairy cows in

Assiut governorate. Veterinary World. 2(10):373-380.

Santos, MV, and DM Barbaro. 2003. Effect of somatic cell count on proteolyses and lipolysis in pasteurized fluid milk during shelf-life storage. Journal of Dairy Science. 86:2491-2503.

Seegers, H, C Flouricho, and F Beaudu. 2003. Related to mastitis economics in dairy cattle herds. Veterinary Research. 34:475-91.

Schukken, YH, DJ Wilson, F Welcome, LG Tikofsky, and RN Gonzalez. 2003. Monitoring udder health and milk quality using somatic cell counts. Veterinary Research. 34:579-596.

Sharma, N, NK Singh, and MS Bhadwal. 2011. Relationship of somatic cell count and mastitis: An overview. Division of Veterinary Clinical Medicine and Jurisprudence, SKUAST-J, India. Asian-Australian Journal of Animal Science. 24(3): 429-438.

Syamsi, AN, M Pratiwi, and AP Nugroho. 2019. Inhibition Activity of Garlic (Allium sativum) Skin Extract on Mastitis Causing Microorganism. Animal Production, 21(1):38-42.

Viguier, C, S Arora, N Gilmartin, K Welbeck, and R O'kennedy. 2009. Mastitis detection: current trends and future perspectives. Trends in Biotechnology. 27. 486-493.

Waminal, YO. 2021. Low-cost liquid anionic surfactant in mastitis detection for small scale dairy buffalo raisers in the Philippines. European Journal of Agricultural and Rural Education. 2(4), 43-48.

Waminal, YO, GASP Tubalinal, and CN Mingala. 2019. Molecular characterization of lactoferrin gene as genetic marker to subclinical mastitis in water buffaloes (Bubalus bubalis). Asian Journal of Agriculture and Biology. 7(4):564-572.

Zdunczyk, S, H Zerbe, and M Hoedemaker. 2003. Importance of oestrogen and oestrogen-active compounds for udder health in cattle: A review. Dtsch Tierarztl Wochenschr. 110: 461.

ISSN 2960-3692

RICE-BASED BIOSYSTEMS JOURNAL

Volume 10 ▪ Issue 1 ▪ February 2022

Philippine Rice Research Institute
Central Experiment Station
Maligaya, Science City of Muñoz, 3119 Nueva Ecija

FULL PAPER

ESTABLISHMENT OF THE CAPILLARY IRRIGATION (*CAPILLARIGATION*) SYSTEM FOR SWEETPOTATO

Joshua D. Mari[1], Mary Grace N. Semilla[1*], and Ricardo F. Orge[2]

[1] Tarlac Agricultural University, Malacampa, Camiling, Tarlac
[2] Philippine Rice Research Institute, Science City of Muñoz, Nueva Ecija
*Corresponding Author: mgnsemilla@tau.edu.ph

Abstract

During extreme drought periods when it is already too risky to plant rice, farmers are usually advised to plant other crops to maximize the use of limited water supply so they can have an alternate income source. Sweetpotato (*Ipomoea batatas*) is commonly planted in rainfed areas or during drought. In this study, the capillarigation system of Philippine Rice Research Institute was tried and evaluated as an alternative irrigation method for sweetpotato production and compared its performance with manual irrigation. The experiment was carried out with four treatments, three of which were represented by the different settings of the *capillarigation* system to effect varying rates of water applications (T_1: 100% depth of riser, T_2: 50% depth of riser, T_3: 25% depth of riser) and the farmers' common practice of manual irrigation serving as the control (T_0). Results showed that the vine length and storage root length and width were not significantly affected by the treatments employed. However, the number of stem vines, storage root weight, and water productivity were significantly influenced. T_1 significantly had the highest average storage root weight of 196.9 kg. On the other hand, water productivity was significantly highest in T_3 with 394.5g harvested root per liter of water used.

Keywords: *Capillarigation System, Climate Change, Drip Irrigation, Drought, El Niño, Irrigation, Sweetpotato*

Introduction

The entire world is experiencing climate change. The World Meteorological Organization (WMO) reported an increasing trend in the global annual mean temperature in the past 45 years. Their recent reports showed that 2020 was one of the three warmest years (2016 as warmest) on record, which is 1.2 ± 0.1 °C above baseline years of 1850 -1900 (WMO, 2021). Climate change has been affecting the lives and livelihood (WMO, 2021) especially in the developing countries (Porio et al., 2018). In the Philippines, the agricultural sector is highly affected. In 2016, for example, there was a decrease in rice production due to typhoon occurrences, dry spell, and drought (PSA, 2018).

Rice production requires a lot of water, estimated to be on the average of 1,432 L per kg of paddy grains produced under an irrigated lowland production system. Thus, planting rice during periods of forecasted El Niño or when there is uncertainty of water supply is risky (Stuecker et al., 2018; Lansigan et al., 2000). Initial investments such as land preparation, seeds, and fertilizer may be wasted if the available water supply is no longer enough to sustain the growth of the rice crop. With this, farmers ought to adapt crop diversification so that their income will not be solely dependent on rice production. Thus, an alternative crop like sweetpotato, which is drought tolerant and requires less water (Siqinbatu et al., 2014) than rice can be planted. Sweetpotato can also substitute rice as staple food (Portilla and Pagaduan, 2014).

Sweetpotato production has high potential of increasing farmers' income. It ranked 5th in terms of value of production with PhP 1.054 million value, next to onion, mango, string beans, and coconut (PSA, 2021b). Central Luzon is the third largest sweetpotato producer in the Philippines with 10.1% share in 546.89 thousand MT produce (PSA, 2021a). In this region, 73.8% of the 2020 sweetpotato production is accounted from Tarlac (PSA, 2021b); thereby, making the province the largest commercial producer of sweetpotato (Pagcaliwangan, 2016).

Although sweetpotato is drought tolerant and can withstand limited water (Siqinbatu, 2014), irrigation is still critical as it affects root yield. Decreased water supply resulted in decreased storage root weight (Ekanayakem and Collins, 2004; Felix et al., 2015) while excessive water also affects root development (Pardales and Yamauchi, 2003). Thus, the need to check water management.

Technologies like drip irrigation is an efficient way of utilizing water (Maisiri et al., 2005; Megersa and Abdulahi, 2015). However, the system needs relatively high initial investment (Ali, 2022; Rowe et al., 2014). Thus, a more economical water efficient system like the capillary irrigation technology or *capillarigation* developed by the Philippine Rice Research Institute (PhilRice), which makes use of capillary wicks (Orge and Sawey, 2019) can be employed.

The system of irrigating plants by capillary action has been adopted in several studies (Semanda, 2018). However, research on this area has not progressed for large scale plant production (Million et al., 2007). The *capillarigation* system showed good performance in green pepper fields (Orge and Sawey, 2019), paving opportunities in exploring its application to other crops.

This study evaluated the applicability of the *capillarigation* system for sweetpotato production. Specifically, it aimed to: (a) set-up the system in plots prepared for planting sweetpotato, (b) determine the volume of water applied in relation to system's setting, and (c) evaluate system performance in terms of the crop's agronomic characteristics and yield parameters.

Materials and Methods

The capillarigation system components

This experiment used the *capillarigation* system developed by the Philippine Rice Research Institute (PhilRice) as a low-cost alternative to drip irrigation of rice-based crops especially when water supply is limited and that planting of rice is no longer possible. Its layout is almost similar to the drip irrigation except that capillary wicks are used as means of dispensing water (Figure 1) instead of the emitter or dripper, which is typically used in the drip irrigation system. It was designed to be a do-it-yourself type of irrigation system maximizing the use of local and recycled materials. More details in the design and setting up of the system are provided in the two publications of Orge and Sawey (2017, 2019).

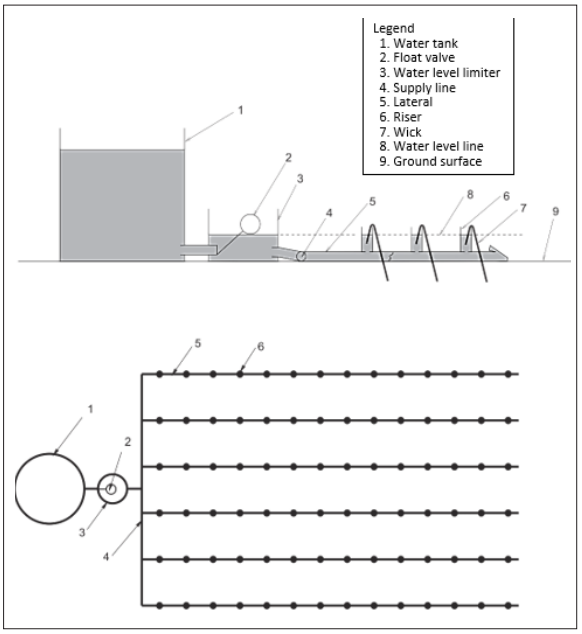


Figure 1. Schematic layout of the *capillarigation* system (Orge and Sawey, 2017)

In this study, a 200 L plastic cylindrical container was used as water tank and a 16 L plastic pail as water level limiter. For the water supply and distribution lines, a 12.5 mm (1/2") diameter PVC pipes cut into desired lengths were used and complemented with PVC tee fittings and elbows. The same size of PVC was also used for the risers, which were individually cut into 18 cm length. Commercially available cotton rope was used as capillary wicks. To minimize water loss due to evaporation, each wick was covered with recycled plastic drinking straw.

Field layout and establishment

This study was conducted in Brgy. Paul, Mangatarem, Pangasinan from August 2020 to June 2021. The field used was idle (fallowed) prior to the setting up of this study. Land preparation was done using 4W tractor-mounted rotavator to remove the weeds and loosen the soil to attain a good tilth suitable for planting.

To create variations in the rate of application of water using the *capillarigation* system, the depth of water relative to the height of the riser varied in this study by changing the location of the float valve relative to the height of the 16 L pail used as water level limiter. This variation resulted incorresponding changes in the freeboard i.e., the difference in height between the riser and the water level inside the riser.

Table 1 presents the treatments of the study. T_1, T_2, and T_3 represent the settings of the *capillarigation* system, which were described in terms of the volume of water inside the water level limiter with its height corresponding to the height of water in the riser. In T_1, for example, the amount of water in the secondary tank is 7 L, which corresponds to a fully filled riser. On the other hand, in T_3, the 2 L volume of water in the secondary tank corresponds to a riser that has only a water height of ¼ (25%) of its height. Manual irrigation was also added in the treatment to serve as the control (T_0) and as basis in comparing the performance of the *capillarigation* system with the existing irrigation practice.

Table 1. Treatment used.

Treatment Code	Description
T_0	Control; manual irrigation
T_1	*Capillarigation* setup; 7 L maximum level; 100% depth of riser (every other day monitoring)
T_2	*Capillarigation* setup; 3 L maximum level; 50% depth of riser (once a week monitoring)
T_3	*Capillarigation* setup; 2 L maximum level; 25% depth of riser (once a month monitoring)

Figure 2 shows a setup of the *capillarigation* system, typical for T_1, T_2, and T_3. As shown, the distance between rows (ridge) was 100 cm and each row had an effective length of 3 m just enough to accommodate 10 hills of sweetpotato spaced at 30 cm between hills. Each hill was planted with 1 cut vine.

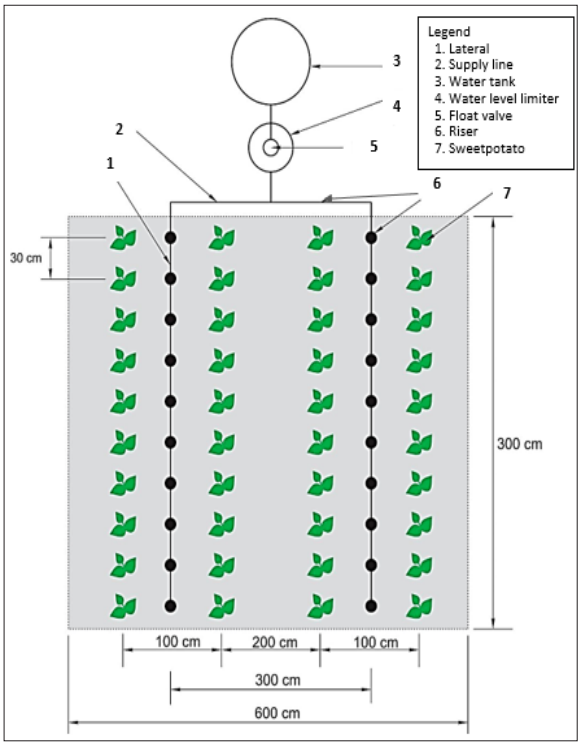


Figure 2. Layout of the *capillarigation* system established for sweetpotato production in the study.

Data gathered

The following data were used in evaluating the performance of the *capillarigation* system (represented at different settings) relative to that of the control (manual irrigation):

1. Volume of water applied. For the plots using the *capillarigation* system, the volume of water supplied to the plants was determined based on the total amount of water added to the initially-filled tank devoted for each treatment. For the control, volume of water was supplied through a plastic pail. The volume of water applied per plant was computed using the formula:

   $V = V_t / n$

   Where:

   V_t = total accumulated volume of water applied throughout the crop growth

   n = number of plants

2. Plant parameters. Data were collected immediately after harvesting. Plant parameters were determined in terms of the following:
   a. length and width of storage roots
   b. length of sweetpotato vines
   c. number of the vine stems
3. Yield. This was determined by taking the root yield of five randomly selected plants representing each treatment.
4. Water productivity. This was computed using the formula:

   $E_{wu} = Y/V_t$

   Where:

   V_t = total accumulated volume of water applied throughout the crop growth.

   Y= total crop yield in the area

Data analysis

Data were analyzed in a randomized complete block design using Statistical Tool for Agricultural Research (STAR). Further analysis was run by STAR for comparison of treatment means using Least Significant Difference (LSD).

Results and Discussion

General information

Figure 3 shows the *capillarigation* system established in plots planted with Super Bureau variety (VSP 6). Aside from being easy to assemble, the system components can easily be purchased in local stores (Appendix Table 1). The sweetpotato planting materials were sourced out from the Rootcrops Research and Training Center based at the Tarlac Agricultural University, Malacampa, Camiling, Tarlac. Two rainfall occurred during the study; however, these were not documented. Sweetpotatoes were harvested after 4 months from the planting date.

Figure 3. The *capillarigation* system installed in sweetpotato plots.

Volume of water supplied

As shown in Figure 4, the highest volume consumed in the whole duration of cropping was T_1 (235 L). This is followed by the control setup with 160 liters. T_2 and T_1 were supplied with 47 L and 8 L for the whole growing season, respectively. It was observed that storage roots were still produced in T_2 and T_3, which indicates that sweetpotato can be grown during scarce water conditions.

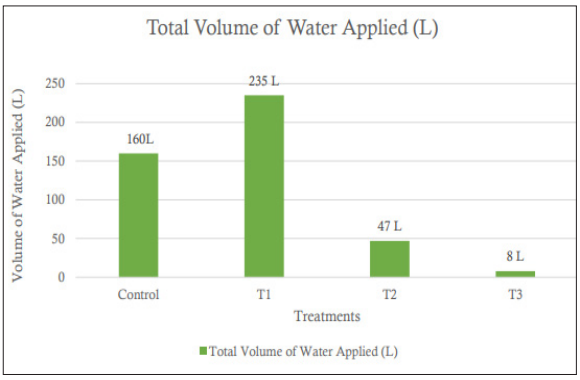


Figure 4. Total volume of water applied in each of the treatments.

Growth parameters

The *capillarigation* system was evaluated based on sweetpotato agronomic characteristics including storage roots yield. Table 2 shows the growth parameters of sweetpotato in terms of vine length and number of vines. T_1 has the longest vine produced, followed by control, T_2 and T_3. However, analysis of variance showed that vine length is not significantly different among treatments. Li et.al (2021) found that more water promotes growth of sweetpotato shoot. Similarly, vine lengthened with increased irrigation (Gajanayake and Reddy, 2016). It can be noted that these studies involved deficit irrigation treatments, which is not the case in this research.

Result of this study agrees with Sokoto and Gaya (2016), which concluded that irrigation interval had no significant effect on the vine length because the supplied water was sufficient enough for vine growth. On the other hand, the average number of vines had significant differences among treatments with T_1 having the most number of vines. This was followed by those plants under control, and then T_2 and T_3 which were not significantly different from each other. Gomes and Carr (2001) showed that vine production is higher in wet season than in dry season. Vine number also increased with more irrigation (Saqib, et.al, 2017). Both the length and number of vines contributed to the vine yield. Furthermore, increasing irrigation frequency caused higher vine yield (Saqib, et al., 2017, Biswal et al., 2017). Above-ground growth can be increased to promote source capacity, which leads to higher vine yield and eventually, high harvest (Li et al, 2021).

Table 2. Growth parameters of sweetpotato under different treatments

Treatment	Vine length, cm	No. of vines
T_0 - Control; Manual Irrigation	196.0	3.8 ab
T_1 - 7 L maximum level; 100% depth of riser	213.8	4.4 a
T_2 - 3 L maximum level; 50% depth of riser	189.0	3.4 b
T_3 - 2 L maximum level; 25% depth of riser	146.6	3.6 b
	ns	*

Yield parameters

Storage root length, storage root diameter, and storage root weight increase with irrigation frequency or shorter irrigation interval (Saqib et al., 2017; Nedunchezhiyan et al., 2012). However, this is not the case for this study's storage root diameter and length (Table 3). All treatments produced roots, which length and width do not significantly differ from each other. This can be due to the insignificant difference in vine length, which directly affects yield parameters (Saqib, et al., 2017). Root length and diameter were higher in longer irrigation intervals; however, root diameter does not significantly vary (Abu El-Fotoh, et al., 2019). Storage root length and width are parameters related to assessing quality of harvest (Bryan et al., 2003; Abd El-Baky et al., 2010; Villordon et al., 2018). All treatments in this study produced relatively similar quality of storage roots while the weight of harvested roots significantly varied (Table 3). Storage roots weight was highest in T_1, followed by control, T_3, and T_2 (Figure 5). This can be attributed to the vine yield in each of the treatment. Increasing irrigation frequency also increased root yield (Nedunchezhiyan et al., 2012), which is supported by the yield result from T_3, T_1, and control irrigation. However, T_3 produced higher storage roots yield than T_2, which was watered more frequently. This could mean that certain irrigation level could affect storage root yield. Almost similar observations were also noted by Thompson, Smittle, and Hall (1992); Gajanayake and Reddy (2016); and Li et al., (2021). This is where water use efficiency or water productivity comes in to assess the resource utilization of a technology (Maisiri et al., 2005; Mergesa and Abdulahi, 2015).

Water productivity

In this study, water productivity was significantly affected by the different treatments (Figure 6). It can be observed that T_3 with a maximum water level of 2 L or 25% depth of riser had significantly highest storage root produced per liter of water supplied with 394.50 g per liter. It is followed by T_2 with 57.28 g of storage roots produced. Control and T_1 produced 20.58 and 17.69 g of storage roots per liter, respectively. While other studies showed that increasing irrigation levels increased water use efficiency (Laurie, Plooy and Laurie, 2009; Mantovani et al., 2013; Zhang et al., 2018), this study matches results gathered by Nedunchezhiyan, Gangandhara, Ray (2012) and Li et al., (2021), who found that water is more efficiently utilized at lower irrigation levels. This may indicate that with less amount of water applied through *capillarigation*, especially in T_3 and T_2, sweetpotato can maximize or save water resource. This also shows that sweetpotato, despite limited water supply, can still be productive (Siqinbatu et al., 2014). It should also be noted that the crop was established during the last two months of rainy season and first 2 months of dry season. Solis et al. (2014) reported that soil moisture status for the first 30 days after transplanting (DAT) affected root development. Despite T_3 having very low amount of irrigation, the rainfall occurrences might have supplied just enough moisture within 30 DAT;

Table 3. Sweetpotato yield parameters of the different treatments.

Treatment	Storage root length (cm)	Storage root width (cm)	Storage root weight (g)
T_0- Control; Manual irrigation	3.49	1.98	164.60 b
T_1 - 7 L Maximum level; 100% depth of riser	4.48	2.40	207.80 a
T_2 - 3 L Maximum level; 50% depth of riser	3.93	1.83	134.60 c
T_3 - 2 L Maximum level; 25% depth of riser	3.31	1.88	157.80 bc
	ns	ns	*

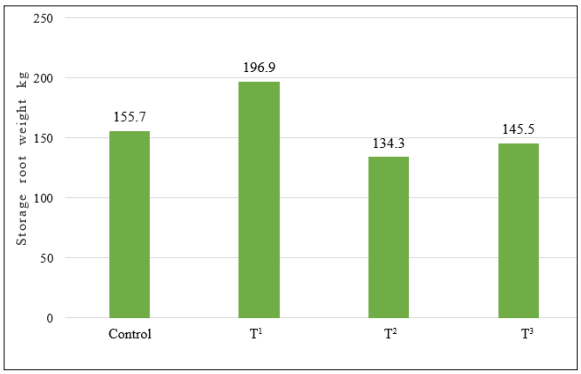


Figure 5. Total actual weight of harvested sweetpotato storage roots from each treatment.

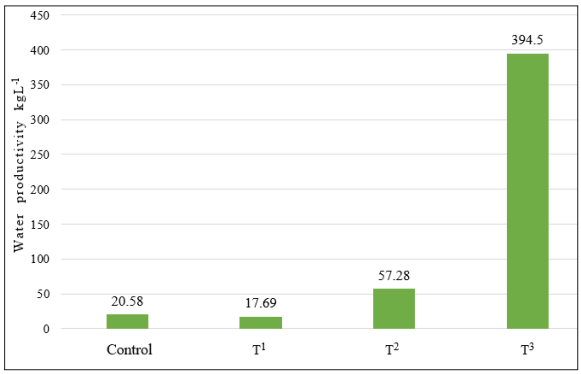


Figure 6. Water productivity of each treatment.

thereby, producing storage roots. Certain genotypes have the ability to be productive amid less water (Solis et al., 2014; Andrade et al., 2016), which could mean that the variety used in this study might also be drought-tolerant. Irrigation method did not affect water use efficiency (Onder et al., 2015). Results from T_1 and control, which employed different irrigation systems, were not significantly different. However, if storage root yield is considered, T_1 showed more advantages.

Conclusion

In this study, the PhilRice-developed *capillarigation* system was used to irrigate plots planted with sweetpotato and compared its performance, measured in terms of the crop's growth (number of vines and vine length) and yield parameters (storage root dimensions and weight), with the traditional, manual method of irrigation. The *capillarigation* system was operated in three settings, each setting representing a treatment, with the manual irrigation as the control. The following conclusion are drawn from the study results:

1. The *capillarigation* system can be an alternative irrigation method for sweetpotato. Under optimum setting, water application is more efficient than the conventional practice of manual irrigation;

2. The adjustments on the water level at the secondary tank, which were represented by the three treatments (T_1, T_2, and T_3), can be a practical way of varying the water application rate. Results show that a 100% level setting (T_1) led to the highest crop yield while the highest water productivity was recorded at 25% (T_3). Setting to be used is based on field condition. T_3 setting could be the best option in areas with limited water. However, if water supply is good, the best option is to target for the highest yield;

3. The installation of the *capillarigation* system may require an added cost. However, this can be compensated by savings in labor cost as once installed, the plots are already self-watering. System components can also be made from recycled materials, which can significantly lower down its investment cost.

Acknowledgment

The researchers are grateful for the assistance of the Director of Rootcrops Research and Training Center in Tarlac Agricultural University, Dr. Lilibeth B. Laranang; members of the advisory committee, Dr. Leonell P. Lijauco and Engr. Jesus A. Tolentino; pieces of advice from Engr. Donna Fay N. Labrador and Dr. Amy Lizbeth J. Rico; and the Bachelor of Science in Agricultural Engineering Class of 2021.

Literature Cited

Abu El-Fotoh HM, Abd-El-Kader AS, Manssur FYO (2019) Effect of irrigation intervals, antitranspirants, compost and humic acid on growth and yield of sweetpotato. Plant Production Science. Zagazig Journal of Agricultural Research **6**(3): 649-664

Ali MH (2022) Practices of irrigation and on-farm water management. Volume 2. New York City: Springer

Andrade MI, Naico A, Ricardo J, Eyzaguirre R, Makunde G, Ortiz R, Gruneberg W (2016) Genotype x environment interaction and selection for drought adaptation in sweetpotato *(Ipomoea batats* L. Lam.) in Mozambique. Euphytica **209**: 261-280

Biswal S, Nedunchezhiyan M, Mohapatra PK (2017) Effect of irrigation schedule and fertilizer levels on growth and yield of sweetpotato (*Ipomoea batatas* L.) Journal of Rootcrops. Indian Society for Rootcrops **43**(1): 44-51

Bryan AD, Schultheis JR, Pesic-VanEsbroeck Z, Yencho GC (2003) Cultivar decline in sweetpotato. II. Impact of virus infection on yield and storage root quality in 'Beauregard' and 'Hernandez'. Journal of the American Society for Horticultural Science **128**(6): 856-863

Ekanayakem I, Collins W (2004) Effect of irrigation on sweet potato root carbohydrates and nitrogenous compounds. Food, Agriculture and Environment **2**(1): 243-248

El-Baky A, Ahmed AA, El-Nemr MA, Zaki MF (2010) Effect of potassium fertilizer and foliar zinc application on yield and quality of sweetpotato. Research Journal of Agricultural & Biological Sciences **6**(4): 386-394

Felix J, Shock C, Ishida J, Feibert E, Saunders L (2015) Irrigation criteria and sweetpotato cultivar performance in the Treasure Valley of Eastern Oregon. HortScience **50** (7): 1011-1017

Gajanayake B, Reddy KR (2016) Sweetpotato responses to mid- and late-season soil moisture deficits. Crop Science **56**(4): 1865

Gomes F, Carr MKV (2001) Effects of water availability and vine harvesting frequency on the productivity of sweetpotato in Southern Mozambique. I. Storage root and vine yields. Experimental Agriculture **37**(4): 523-537

Lansigan FP, delos Santos WL, Coladilla JO (2000) Agronomic impacts of climate variability on rice production in the Philippines. Agriculture, Ecosystems & Environment **82**(1-3), 129-137

Laurie RN, Plooy DU, Laurie SM (2009) Effect of moisture stress on growth and performance of orange fleshed sweetpotato. In *African Crop Science Conference Proceedings* **9:** 235-239

Li S, Zhao L, Sun N, Liu Q, Li H (2021) Photosynthesis product allocation and yield in sweepotato with different irrigation levels at mid-season. Agricultural Water Management **246**: 10678

Mantovani EC, Delazari FT, Dias LE, Assis IR, Vieira GH, Landim FM (2013) Yield and water use efficiency for two sweetpotato cultivars depending in irrigation depths. HorticulturaBrasileira **31**(4)

Maisiri N, Senzanje A, Rockstrom J, Twomlow SJ (2005) On farm evaluation of the effect of lowcost drip irrigation on water and crop productivity compared to conventional surface irrigation system. Physics and Chemistry of the Earth, Parts A/B/C **20 (11-16)**: 783-791

Megersa G, Abdulahi J (2015) Irrigation system in Israel: A review. International Journal of Water Resources and Environmental Engineering **7**(3): 29-37

Million J, Yeager T, Larsen C (2007) Water use and fertilizer response of *Asalea* using several no-leach irrigation methods. Hortechnology **17**(1): 21-25

Nedunchezhiyan M, Gangadharan B, Ray RC (2012) Effect of tillage, irrigation, and nutrient levels on growth and yield of sweet potato in rice fallow. International Scholarly Research Network. ISRN Agronomy 2021

Onder D, Onder S, Caliskan ME, Calikskan S (2015) Influence of different irrigation methods and irrigation levels on water use efficiency, yield, and yield attributes of sweet potatoes. Fresenius Environmental Bulletin **24**(10a): 3398-3403

Orge RF, Sawey DA (2017) Coping with water scarcity in rice-based farms: development of a capillary irrigation system for smallholder farmers in the Philippines. In *Gorawala, P. and Mandhatri, S.* Agricultural Research Updates **16**: 191-207

Orge RF, Sawey DA (2019) Field performance of the capillary wick irrigation (capillarigation) system for rice-based crops. International Journal of GEOMATE **17**(61): 41-49

Pagcaliwangan B (2016) Sweetpotato fiesta held in Camiling Tarlac. Retrieved from http://www.pcaarrd.dost.gov.ph/home/portal/index.php/quick-information-dispatch/2697-sweetpotato-fiesta-held-in-camiling-tarlac (accessed February 18, 2022)

Pardales JR, Yamauchi A (2003) Regulation of root development in sweetpotato and cassava by soil moisture during their establishment period. Plant and Soil **255**: 201-208

PSA [Philippine Statistics Authority] (2018) Crops statistics of the Philippines 2016-2020. Retrieved from https://psa.gov.ph/content/crops-statistics-philippines-national-and-regional (accessed February 18, 2022)

PSA [Philippine Statistics Authority] (2021a) Crops statistics of the Philippines 2016-2020. Retrieved from https://psa.gov.ph/content/crops-statistics-philippines-national-and-regional (accessed February 18, 2022)

PSA [Philippine Statistics Authority] (2021b) Central Luzon's total value of other crops value and volume of production in 2020. Retrieved from http://rsso03.psa.gov.ph/article/central-luzons-total-value-other-crops-value-and-volume-production (accessed February 18, 2022)

Porio E, Dator-Bercilla J, Narisma G, Cruz F, Yulo-Loyzaga A (2018) Drought and urbanization: The case of the Philippines. In *Urban Drought* (pp 183-208). Singapore: Springer

Portilla JM, Pagaduan JMR (2014) Status of other staple crops as substitute to rice: an assessment in Isabela and Quirino [Philippines]. Philippine Journal of Crop Science. **39**: 132-133

Rowe BD, Kolp MR, Greer SE, Getter KL (2014) Comparison of irrigation efficiency and plant health of overhead, drip, and sub-irrigation for extensive green roofs. Ecological Engineering **64**: 306-313

Saqib M, Khalid M, Hussain S, Anjum M (2017) Effect of water stress and planting system on growth yield and quality of sweet potato. Acta Scientiarum Polonorum Hortorum. Cultus **16** (6): 201-210

Semanda NPK, Ward JD, Myers BR (2018) A semi-systematic review on capillary irrigation: the benefits, limitations, and opportunities. Horticulturae **4**(3):23

Siqinbatu YK, Hirai H, Shibuya T, Endo R (2014) Effects of soil water content on the growth and yield of sweetpotato grown on sandy soil. Eco-Engineering **26**(3): 75-80

Sokoto MB, Gaya MI (2016) Growth and yield of sweetpotato (*Ipomoea batatas* L.) as influenced by irrigation interval and variety in Sokoto Sudan Savananah, Nigeria. International Journal of Plant and Soil Science **11**(3):1-12

Solis J, Villordon A, Baisakh N, LaBonte D, Firon N (2014) Effect of drought on storage root development and gene expression profile of sweetpotato under greenhouse and field conditions. Journal of the American Society for Horticultural Science **139**(3): 317-324

Stuecker M, Tigchelaar M, Kantar M (2018) Climate variability on rice production in the Philippines. PLoS ONE **13**(8): 0201426

Thompson PG, Smittle DA, Hall MR (1992) Relationship of sweetpotato yield and quality to amount of irrigation. HortScience **27**(1): 23-26

Villordon A, Gregorie JC, LaBonte D, Khan A, Selvaraj M (2018) Variation in 'Bayou Belle' and 'Beauregard' sweetpotato root length in response to experimental phosphorous deficiency and compacted layer treatments. HortScience **53**(10): 1534-1540

WMO [World Meteorological Organization] (2021) State of global climate 2020. Retrieved from https://public.wmo.int/en/our-mandate/climate/wmo-statement-state-of-global-climate (accessed February 23, 2022)

Zhang H, Xie B, Dong S, Wang B, Zhang L, Shi C (2018) Effects of different periods of drought stress on photosynthetic efficiency and water consumption characteristics of sweet potato. Journal of Applied Ecology **29**(6): 1943-1850

Annex Table 1. Cost of materials for the capillarigation setup (3 treatments).

Materials	Quantity/Unit	Unit Price	Actual Cost (PhP)
Plastic drum (200 L)	3 pcs	700	2,100.00
Plastic pail (16 L)	3 pcs	100	300.00
Plastic floater	3 pcs	50	150.00
PVC pipes, 12.5 mm (1/2 in) x 3m	20 pcs	20	400.00
Cotton rope	1 roll	50	50.00
Plastic faucet	3 pcs	20	60.00
Plastic drinking straw	1 pack	20	20.00
PVC tee fittings	35 pcs	10	350.00
Sealants	2 tubes	35	70.00
Elbows, 12.5 mm (1/2 in)	9 pcs	10	90.00
Material Cost			3,590.00
Labor Cost			1,200.00
Total Cost			4,790.00

Prevalence of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* Antibodies in Free Range Chickens as Detected by ImmunoComb Assay

ANNALIE B. PARAGAS AND MA. ASUNCION G. BELTRAN

College of Veterinary Medicine, Tarlac Agricultural University
Camiling, Tarlac, Philippines
abparagas@tau.edu.ph

Abstract

This study was conducted to detect the antibody titer *Mycoplasma gallisepticum* and *M. synoviae* on the free-range chicken project at the Tarlac Agricultural University and its beneficiaries in Santa Ignacia. A total of 110 blood samples from free-range chickens were collected, regardless of age, sex, and breed. These samples were tested for Mycoplasma infection using the ImmunoComb Assay.

Results showed that 56.4% were found positive for *Mycoplasma synoviae* and 46.3% positive for *Mycoplasma gallisepticum*. However, even though they were found positive, the antibody titer for *Mycoplasma synoviae* was only 1.82 ImmunoComb® unit in Tarlac Agricultural University – FRC Project, and 1N.34 ImmunoComb® unit in Santa Ignacia FRC beneficiaries. This finding indicate weak positive. Further, Mycoplasma gallisepticum with 1.40 ImmunoComb® unit in Tarlac Agricultural University – FRC Project, and 1.11 ImmunoComb® unit in Sta Ignacia FRC beneficiaries were found to be weakly positive. Based on the results of the study, the *Mycoplasma gallisepticum* and *Mycoplasma synoviae* tested were both prevalent but in low antibody titers on the Free-

range Chicken project at Tarlac Agricultural University and its beneficiaries in Santa Ignacia.

Keywords. *Antibody, Chicken*, *ImmunoComb®, Mycoplasma gallisepticum, Mycoplasma synoviae*

INTRODUCTION

Free-range chicken production nowadays is highly in demand as free-range chickens are believed to be good sources of nutritious meat and eggs for the consumers. The management system of production of free-range chickens is more like native chickens for they range in open fields for acquiring their food. Through this system of production, the chickens are highly susceptible to Mycoplasma infections.

One of the most serious diseases that affect the avian species is caused by Mycoplasma. Mycoplasma species that are most important in causing diseases in poultry farming are *M. gallisepticum, M. meleagridis,* and *M. synoviae*. The condition occurs worldwide and affects the production and performance of poultry farms. In some countries, this infection occurs rarely in commercial poultry.

In previous studies, Mycoplasma causes chronic respiratory disease in chickens and sinusitis in turkeys. Mycoplasma disease is characterized by respiratory rales, coughing, nasal discharge and conjunctivitis, and infraorbital sinusitis in turkeys. Increased carcass and downgrading condemnation caused by aereosacculitis, decreased growth and egg

production, and increased medication costs, make MG one of the costliest infection diseases (Raviv and Ley, 2013). Mycoplasma infection induces significant economic losses in poultry by reducing body weight gain, meat quality, and feed conversion rate in broilers, causing a significant decline in egg output in layers, and increasing embryo mortality in breeders. Blood and serum testing kits are used to determine the antibody titers of *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) in chicken flocks. ImmunoComb assay is a kit that detects the antibody of infection of Mycoplasma spp in chickens, this is used to diagnose the presence of the bacteria causing the low production of chickens.

Hence, this study was conducted to determine the presence of Mycoplasma in Free Range Chickens at Tarlac Agricultural University and its beneficiaries in Santa Ignacia.

METHODOLOGY

Management of Experimental Animals

A total of 110 free-range layer chickens aged five to eight months, both sexes, with an average of 120 kgs body weight, was used in the study**.** The experimental animals were housed at the Free-Range Chicken Project, Tarlac Agricultural University, and in the residents of the beneficiaries of the project in Sta. Ignacia, Tarlac. The chickens were semi-confined and were ranged in the morning to exercise and exhibit their natural behaviors. They were fed with commercial feeds added with forages in the morning and the afternoon. Water was also provided daily.

Vaccination and Health management

The chickens were vaccinated at the age of two weeks with the New Castles Disease vaccine B1B1. The vaccine was administered intraocular, one drop of NCD B1B1 in the eye per bird. Then they were administered with LaSota Newcastle disease vaccine at four weeks of age. In breeder and layer chicken flocks, the vaccine needs to be repeated at 3-month intervals to maintain a sufficient level of immunity. At the age of 18 weeks, the chickens were vaccinated with the NCD vaccine for further immunity, blood collection, and for the Immunocomb assay.

Biogal's ImmunoComb

Biogal" ImmonoComb Assay is a diagnostic test for specific antibodies in the animals' blood. The kit is similar to ELISA or Enzyme Link Immunosorbent Assay principle, which is used to test specifically Mycoplasma infection in chickens. This is produced by Biogal Galed Labs., Gale Kibbutz, Israel.

Development Process of Assay (Biogal's ImmunoComb)

Using paper disk

By using a paper disk, one of the chicken's veins was pierced. Then a specimen paper was taken and saturated a pre-punched disk with the blood. Using tweezers, the protective aluminum cover of wells in row A was slit open. Then a disk saturated with blood was punched out. This was followed by inserting the disks into the diluents which was succeeded by the extraction of antibodies. After that, next 2 consecutive wells for the control serum were opened. A 5 µl Positive Control Serum (C+) was taken and inserted

into well A next to the last sample. The serum was mixed into the well. Then the same steps were done with the Negative Control Serum (C-) in the following well. After that, one comb was removed from its protective wrapping and was inserted (printed side facing you) into Row A. Then, it was incubated for 10 minutes. To improve mixing, the researcher gently moved the Comb up and down at the start of each incubation (each row). This was repeated at least twice in all of the remaining rows. The cover of wells was pierced in Row B with the tweezers. The excess liquid was gently shaken off onto a tissue (follow the same procedure for the remaining rows at the end of each step). The comb was inserted into wells of row B and incubated for 2 minutes. The Comb was placed in Row D for 2 minutes, Row E for 2 minutes, and Row F for 10 minutes, allowing the color reaction process to develop. After the Comb had completed the cycle for Row F, it was transferred back to and incubated at Row E for two minutes for color fixation.

Reading Results with the Comb scale

When the comb was completely dry, it was aligned with the calibrated color CombScale provided in the kit. The tone of the comb with the purple-grey on the CombScale closely matches the Positive Control spot the most. Then, the yellow ruler was slid until the C+ mark appeared in the window above the color. The researcher held the slide in this position during the entire reading. In this step, it calibrated the C+ to S3, which was the “cut-off” point to which test spots were compared. The spots were read separately. After that, the researcher chose the most suitable color and read the titer in the yellow windows.

Reading and Interpreting the Results

The middle spot tested for MG and the lower spot tested for MS. The results were evaluated with each disease separately. MG and MS IgG levels were determined by comparing each specimen's color intensity to the Positive Control (C+). Specimens with identical or higher color intensity than the Positive Control were considered positive. The Negative Control consisted of non-immune sera and was read as zero (S=0). Non-specific reactions around S1 (i.e. false positive) occurred occasionally due to various reasons and may be associated with the use of certain commercial vaccines. To avoid misinterpretation of non-specific reactions and possible confusion with true low positive results, it is recommended to confirm results by retesting at a one-week interval. A test color darker than S6 indicates either an acute disease or a highly immune flock.

The Analysis of ImmunoComb® results using CombScore™ sheet

The number of samples was multiplied in a column by the corresponding S value. The answers for each column (S1, S2, etc.) were written in the open box under the column. The numbers (from the previous computation) were added to all the boxes and the sum was written in the total box. The total (from the previous computation) was divided by the sample size (number of birds tested) to arrive at the score. The score was the mean antibody titer of the test sampling.

Data Gathered

The following data were gathered in this study: the number of positive and negative results in detecting *Mycoplasma* infection using the ImmunoComb® Assay, the antibody titer of the test subjects that was found positive, and the mean antibody titer on the free-

range chicken project at the Tarlac Agricultural University and FRC beneficiaries in Sta Ignacia, Tarlac.

Results and Discussions

Detection of *Mycoplasma* infection using ImmunoComb® Assay

The result of the detection of *Mycoplasma* infection using ImmunoComb® Assay on Tarlac Agricultural University – Free-Range chicken (TAU-FRC) project and FRC beneficiaries in Sta Ignacia is shown in Tables 1 and 2. The results were based on the purple-gray color intensity seen on each comb card as shown in Figure 1.

Mycoplasma gallisepticum testing using ImmunoComb® Assay with their respective origin

Table 1 shows the summary results of *Mycoplasma gallisepticum* testing using ImmunoComb® Assay. A total of 46.3% or 51 samples were found positive and 53.7% or 59 samples were found negative with *Mycoplasma gallisepticum* from a total population of 110 free-range chickens from TAU and beneficiaries in Santa Ignacia.

Table 1. Summary of the results in Mycoplasma gallisepticum testing using ImmunoComb® Assay with their respective origin

Developing plate no.	MG n (+)	% in DP	Subtotal n (%+)	MG n (-)	% in DP	Subtotal n (%-)
1	3	30	2.7	7	70	6.4
2	0	0	0	10	100	9.1
3	10	100	9.1	0	0	0
4	10	100	9.1	0	0	0
5	2	20	1.8	8	80	7.3
6	0	0	0	10	100	9.1
7	4	40	3.6	6	60	5.4
8	7	70	6.4	3	30	2.7
9	3	30	2.7	7	70	6.4
10	2	20	1.8	8	80	7.3
11	10	100	9.1	0	0	0
TOTAL	**51**		**46.3**	**59**		**53.7**

Legend: MG n (+) – the number of positive samples of M. gallisepticum

Table 2 shows the summary of the results in *Mycoplasma synoviae* testing using ImmunoComb® Assay. A total of 56.6% or 62 samples out of 110 were found positive, while 43.6% or 48 samples were found negative.

The results showed that all experimental animals were healthy before blood collection, with no visible clinical signs of either *M. gallisepticum* or *M. synoviae*. This supported the claims of Seifi and Shirzad (2011). The absence of clinical disease in chickens in the early stage of *Mycoplasma* was also previously recorded by Levinsohn *et al.* (1989). The study by Talkington *et al.* (1985) stated that it was not uncommon for birds with mild or inapparent clinical signs to be infected with *Mycoplasma*. Ley (2003) also observed that the absence of visible clinical signs could happen even if serologic evidence were recorded. This was when the case had been encountered at a young age

and the chicken could have been partially recovered. The variation in seroprevalence of mycoplasmosis in poultry birds might be due to the replacement of breeding stock with the progeny of the same flock, seasonal influence, poor ventilation, contamination of litters, and no restriction on their movement of the caretaker, visitors, and such other persons as well as other biosecurity measures (Ombase *et al.*, 2018).

Table 2. Summary of the results in Mycoplasma synoviae testing using ImmunoComb® Assay with their respective origin

Developing plate no.	MG n (+)	% in DP	Subtotal n (%+)	MG n (-)	% in DP	Subtotal n (%-)
1	1	10	0.9	9	90	8.1
2	0	0	0	10	100	9.1
3	10	100	9.1	0	0	0
4	10	100	9.1	0	0	0
5	9	90	8.2	1	10	0.9
6	3	30	2.7	7	70	6.4
7	7	70	6.4	3	30	2.7
8	8	80	7.3	2	20	1.8
9	2	20	1.8	8	80	7.3
10	2	20	1.8	8	80	7.3
11	10	100	9.1	0	0	0
TOTAL	**62**		**56.4**	**48**		**43.6**

Legend: *MS n (+) – the number of positive samples of M. synoviae*
MS n (-) – the number of samples negative samples of M. synoviae

This study proves that *Mycoplasma gallisepticum* and *Mycoplasma synoviae* were present in the free-range chicken project at Tarlac Agricultural University and its beneficiaries in Sta Ignacia with seropositivity of 46.3% and 56.4%, respectively. In addition, this study also suggests that even though there were no clinical signs seen in those chickens tested, they could still have *Mycoplasma* infection.

Figure 1 shows that Sample 1.1 is positive for *M. gallisepticum* by the Comb Scale (Figure 2) that measured the antibody titer based on the intensity of the purple-gray spot present in the Combcard. The C+3 block, as seen in Figure 2, was aligned with the control serum (the squared portion in Figure 1). After which, the comb scale was placed at the top of the comb card number 1 for the interpretation of the purple-gray spot precipitated. Still, in Figure 1, the CombScale gave a reading of 4 ImmunoComb® units (S4) for sample 1.1 for *M. gallisepticum* and one (1) ImmunoComb® unit (S1) for *M. synoviae*. Aside from the aforementioned sample number, samples 1.7 and 1.8 yielded two (2) ImmunoComb® units and one (1) ImmunoComb® unit, respectively, both in *M. gallisepticum*.

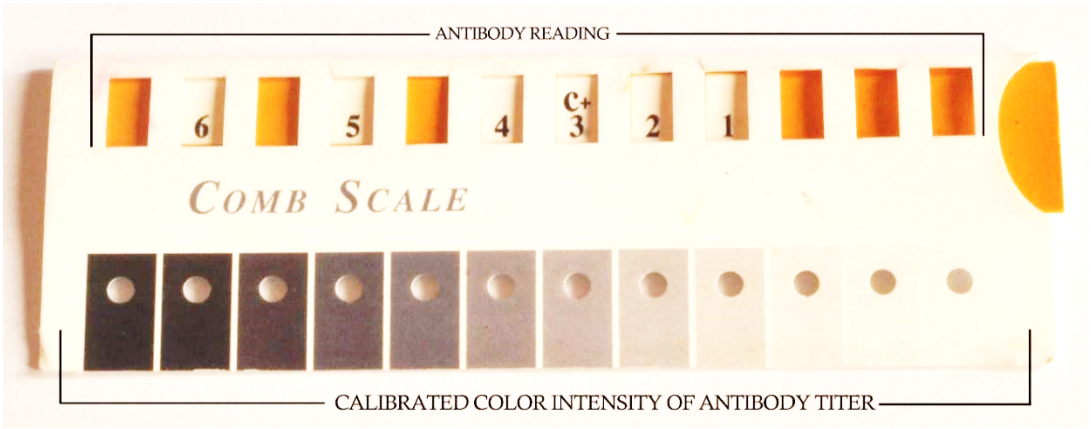


Figure 1. Comb card number 1, shows the purple-gray color results on each comb that corresponds to an antibody titer, the positive and negative control.

Detection of the antibody titer of *Mycoplasma gallisepticum* and *Mycoplasma synoviae*

The result of the detection of antibody titer of *Mycoplasma* infection using ImmunoComb® Assay in Tarlac Agricultural University – Free-Range Chicken (TAU-FRC) project and FRC beneficiaries in Sta. Ignacia is shown in Tables 3 to Table 6.

Antibody Titer for Mycoplasma gallisepticum in TAU- FRC Project

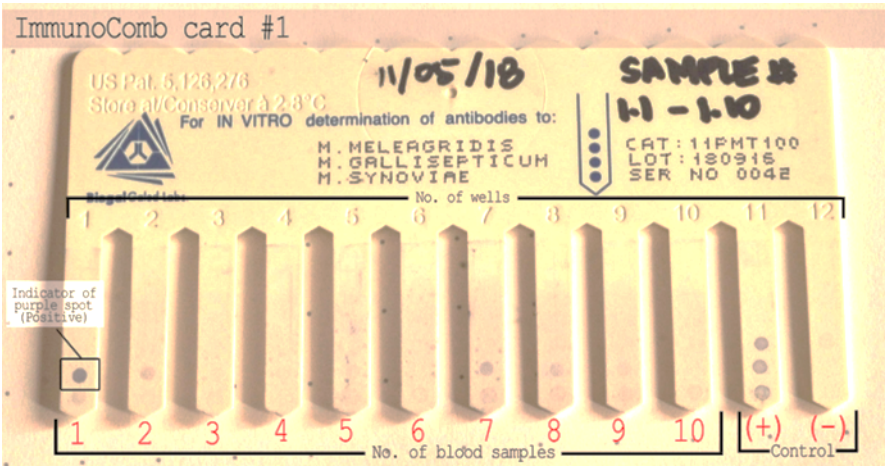


Figure 2. The Combscale shows the different color intensities that correspond to a particular antibody titer or level for each test.

Table 3 shows the summary of antibody titer for *M. gallisepticum* in 60 whole blood samples from the chickens of the TAU-FRC project. Developing plate number one scored 0.7 ImmunoComb® unit (S0.7) which was the fourth to the lowest titer recorded, i.e., Developing plates numbers 2 and 6, which yielded 0 ImmunoComb® units, respectively. Developing plate number 3 scored a 3.7 ImmunoComb® unit (S3.7) which was the highest of all the six (6) developing plates. Developing plates numbers 4 and 5 with 3.6 ImmunoComb® unit (S3.6), and 0.4 ImmunoComb® unit (S0.4), respectively. From the 60 whole blood tests for *Mycoplasma gallisepticum*, the mean antibody titer recorded was 1.40 ImmunoComb® unit (S1.40).

Table 3. Summary of antibody titer for *Mycoplasma gallisepticum* in TAU- FRC Project

Animal Samples	Developing Plates					
	1	**2**	**3**	**4**	**5**	**6**
1	4	0	4	4	0	0
2	0	0	4	4	2	0
3	0	0	4	4	2	0
4	0	0	4	4	0	0
5	0	0	4	3	0	0
6	0	0	4	4	0	0
7	2	0	3	3	0	0
8	1	0	4	3	0	0
9	0	0	3	4	0	0
10	0	0	3	3	0	0
MEAN	0.7	0	3.7	3.6	0.4	0
Mean Antibody Titer	1.40					

Antibody Titer for Mycoplasma gallisepticum in FRC Beneficiaries in Santa Ignacia, Tarlac

Table 4 shows the summary of antibody titer for *M. gallisepticum* with 50 whole blood samples of FRC beneficiaries in Sta. Ignacia, Tarlac. Developing plate number 2 scored the highest mean of 2.2 ImmunoComb® unit (S2.2); second highest was developing plate number 6 with a mean score of 1.8 ImmunoComb® unit (S1.8); followed by developing plate number 1 with a mean score of 1.2 ImmunoComb®unit (S1.2). Developing plates numbers 3 and 4 had the lowest mean score of 0.3 ImmunoComb® unit (S0.3) and 0.03 ImmunoComb® unit (S0.03), respectively. A total mean antibody titer of 1.11 ImmunoComb® unit (S1.11) was recorded for the 50 blood samples of chickens from Santa Ignacia.

Table 4. Summary of antibody titer for *Mycoplasma gallisepticum* in FRC Beneficiaries in Santa Ignacia, Tarlac

Animal Samples	Developing Plates				
	1	**2**	**3**	**4**	**5**
1	4	4	1	2	2
2	5	2	1	1	1
3	0	0	1	0	2
4	0	4	0	0	2
5	0	4	0	0	1
6	1	4	0	0	1
7	2	1	0	0	2
8	0	0	0	0	2
9	0	0	0	0	3
10	0	3	0	0	2
MEAN	1.2	2.2	0.3	0.03	1.8
Mean Antibody Titer			1.11		

Tables 3 and 4 show the summary of antibody titer for *Mycoplasma gallisepticum* with a total mean antibody score of 1.40 in the TAU-FRC project and 1.11 in Santa Ignacia. Though the antibody scores were low, presence of *the Mycoplasma gallisepticum* were detected with these healthy chickens. This finding was answered by Haghighi-Khoshkhoo et. al. (2011) who stated that the seroprevalence of *M. gallisepticum* in the Centernorth of Iran was low; only 4 of 40 (10%) flocks were positive. Malaysia and Burnham et.al (2003) observed that chickens produced good quality eggs and showed good performance although they harbored *M. gallisepticum* organism, despite having a high *M. gallisepticum* antibody. Other sources of infection of the *M. gallisepticum* may be from other birds that enter the farms. This was reported by Tan et. al (2016) in which free-flying birds in close contact with infected chickens may re-transmit the infection when in close contact with commercial chickens and also through fecal sheds.

Low infection of the *M. gallisepticum* in terms of age, in which experimental animals were from 5 to 8 months, was also observed in a similar report confirmed by Hossain et.al. (2007) and Talha (2003) who stated that the prevalence of *M. gallisepticum* infection significantly decreased with the increase of age. The highest infection in young chickens was due to the vertical transmission of the organisms.

In terms of breeds, all breeds of chickens are susceptible to *M. gallisepticum* infection. The prevalence varied widely among different breeds of chickens. These differences might have happened due to breed variation, the nature of poultry farming, operational practices, and other biosecurity measures of the farms (Ali et. al., 2015).

Another factor of infection is the size of the flock which can influence of infection of *M. gallisepticum* which corroborates the observation of Ali et.al. (2015) that the highest infection rate (69.63%) was tested in a large-scale flock (3,000 to 4,200 birds) compared to a small scale (1,300 – 1,600 birds). And this was also detected in the previous investigation of Heleili et. al. (2012) which documented 76.97% of MG infection in a herd containing 18000 birds from 20% in a herd with 500-1000 birds in Algeria. Hossain et.al (2007) stated that *the M. gallisepticum* infection rate was the highest (68.5%) in large flocks compared to small flocks (50.1%) in Rajshahi and surrounding districts of Bangladesh. Though the number of a flock in the TAU-FRC project and Sta Ignacia was on a small scale, *M. gallisepticum* infection might occur.

Summary of Antibody Titer for *Mycoplasma synoviae* in TAU- FRC Project

Table 5 shows the summary of antibody titer for *M. synoviae* in the same 60 whole blood samples from chickens of the TAU-FRC project. Developing plate number 1 scored 0.1 ImmunoComb® unit (S0.1) second to the lowest titer recorded. Developing number 2 yielded 0 ImmunoComb®unit. Developing plate number 3 scored a 3.9 ImmunoComb® unit (S3.9) which was the highest of all the six (6) developing plates tested for *Mycoplasma synoviae*. Developing plates numbers 4, 5, and 6 yielded 3.3 ImmunoComb® unit (S3.3), 3.3 ImmunoComb® unit (S3.3), and 0.3 ImmunoComb® unit (S0.3), respectively. From the 60 whole blood tested for *M. synoviae*, the mean antibody titer recorded was 1.82 ImmunoComb® unit (S1.82)

Summary of Antibody Titer for *Mycoplasma synoviae* from FRC Beneficiaries in Santa Ignacia, Tarlac

Table 6 shows the summary of antibody titer for *Mycoplasma synoviae* from FRC beneficiaries in Santa Ignacia, Tarlac. Developing plate number 2 scored the highest titer with 2.3 ImmunoComb® unit (S2.3), followed by developing plate number 5 with 2 ImmunoComb® unit (S2.0) and developing plate number 1 with 1.7 ImmunoComb® unit (S1.7). Developing plates numbers 4 and 3 had the lowest titer with 0.5 ImmunoComb® unit (S0.5) and 0.3 ImmunoComb® unit (S0.3), respectively. For the whole 50 blood samples, the mean antibody titer was 1.34 ImmunoComb® unit (S1.34) from Santa Ignacia FRC beneficiaries.

Table 5. Summary of antibody titer for *Mycoplasma synoviae* in TAU- FRC Project.

Animal Samples	Developing plates					
	1	**2**	**3**	**4**	**5**	**6**
1	1	0	3	2	2	0
2	0	0	4	3	3	1
3	0	0	4	3	3	1
4	0	0	4	3	3	1
5	0	0	4	3	3	0
6	0	0	4	3	3	0
7	0	0	4	4	4	0
8	0	0	4	4	4	0
9	0	0	4	4	4	0
10	0	0	4	4	4	0
MEAN	0.1	0	3.9	3.3	3.3	0.3
Mean Antibody Titer			1.82			

Table 6. Summary of antibody titer for *Mycoplasma synoviae* from FRC Beneficiaries in Santa Ignacia, Tarlac

	Developing Plates				
Animal Samples	**1**	**2**	**3**	**4**	**5**
1	4	5	1	3	2
2	5	2	0	2	1
3	0	1	0	0	2
4	0	4	0	0	2
5	1	4	0	0	2
6	2	3	0	0	2
7	2	3	0	0	2
8	0	0	0	0	1
9	3	1	0	0	3
10	1	0	1	0	3
MEAN	1.7	2.3	0.2	0.5	2
Mean Antibody Titer			1.34		

A low score of antibody titer results in Tables 5 and 6 of TAU-FRC Project and Santa Ignacia was related to the study of Feberwee (2008) in Dutch commercial farms

that *Mycoplasma synoviae* positive-farms seroprevalence was suggestively lower in layer type of chickens than in meat-type chicken because of the voluntary *M. synoviae* monitoring program aimed at detecting *M. synoviae* infection as early as possible. This was corroborated by Cortes et. al. (2021) who stated that the difference between the system of production of broiler and layer chickens' vaccination against Mycoplasma infection was more common in broiler chickens than in layer chickens. Therefore, reports of vaccination in layer chickens have not been studied. Kleven (1998) stated that the positivity of *M. synoviae* in chickens may often result in mild or even subclinical disease. Sui et al (2021) reported that some *M. synoviae*-infected chickens displayed no clinical signs, which led to the spread of *M. synoviae*, thereby increasing the probability of infection and coinfection with other pathogens.

In contrast with the results of this study, Cortes et. al. (2021) found that layer chickens had a high seroprevalence of *M. synoviae* with 95% and 74% in broiler chickens. Parallel with the results observed by Kapetanov et al. (2010), *M. synoviae* had high seroprevalence rates in adult flocks (90%) and flocks during the rearing period (40%) in 2009 in Serbia. Conversely, another previous investigation stated that lower seroprevalences in commercial layers of 69% (Buim et al., 2009) and 53% (Suzuki et al., 2009) were measured by ELISA.

Results seen in Tables 3 to 6 also showed that there was variability in the serological response of each chicken toward *Mycoplasma* infection. This supported the claim of Kleven (1998) who stated that *Mycoplasma gallisepticum* strains have recently been shown to have the ability to vary the expression of major surface antigens, thus expressing a continually changing "antigenic profile" to the immune system. In addition,

continual variability in the expression of such surface antigens also occurs *in vivo* and may be a major factor in the development of clinical disease and serological responses. Meaning, even with a strong immune response, it is most likely that *Mycoplasma* could still exist because of that variability. It may also help to explain “atypical” serological reactions found in infected flocks.

Razin et al. (1998) stated that Mycoplasma may not be recognized by the host immune system due to its intracellular location during its latency period. Mycoplasma will only induce disease after the host was affected by other disease-causing agents or an episode of host weakness.

Studies of the prevalence of mycoplasmosis in backyard chickens by Haesendonck et al. (2014) and Derksen et al. (2018) showed that the backyard poultry flocks would possibly act as reservoirs or amplifiers for poultry respiratory diseases serving as a continuous source of infection for industrial chickens. Viviana et. al. (2020) detected the presence of *M. gallisepticum* and *M. synoviae* in backyard poultry farms, confirming the potential role of this type of breeding to spread pathogens to commercial poultry production, especially in densely poultry-populated areas where backyard and commercial farms are close. And the result of their study in which all flocks tested were Mycoplasma positive as detected using PCR, suggested that backyard chickens should be tested periodically to determine the status of mycoplasma infection. Therefore, routine monitoring is essential to evaluate the immune status of a flock over time.

Vaccination Program against *Mycoplasma* infection

The decision to vaccinate or simply accept performance losses in commercial layers depended on several factors. The strain of *M. gallisepticum* in a farm must be taken into account as some strains of *M. gallisepticum* were mild while others were highly virulent. According to Butcher (2015), house construction was a major factor in knowing the severity of clinical disease; those layers that were kept in a closed-type house with poor ventilation would experience considerable performance losses. Also, flocks that were placed in open-sided houses and closed houses with excellent ventilation would not experience considerable performance losses. Thus, consideration of air quality where the layers will be housed before vaccination is a must.

Available live vaccines for *M. gallisepticum* were produced from the F strain, and more recently, strains ts-11 and 6/85, which were apathogenic with improved characteristics (OIE, 2012). Administration of the F strain by the intranasal or eyedrop route was preferred, but aerosol or drinking water administration may be used. The eyedrop method is recommended for ts-11, and a fine spray for 6/85. Pullets are generally vaccinated between 12 and 16 weeks of age. One dose is sufficient and vaccinated birds remain permanent carriers (Evans *et al.,* 2005).

Conclusions

Based on the results, *Mycoplasma gallisepticum* and *Mycoplasma synoviae* were both present on the Free-range chicken project at Tarlac Agricultural University and those in the residents of its beneficiaries in Sta Ignacia. The mean antibody titer of the whole test subjects results in *M. gallisepticum* and *M. synoviae* in the TAU-FRC project and its

beneficiaries in Santa Ignacia scored low, this means that under the interpretation given by the manufacturer of the test kit, the antibody titer or level was low or almost undetectable. This might be because the Mycoplasma infection present in the Tarlac Agricultural University – Free-range chicken project and Santa Ignacia, Tarlac was latent or the occurrence of the infection was still in its early course and have not yet severely progressed.

Acknowledgments

The authors would like to thank the following for the success of this study: Tarlac Agricultural University for funding this study, the Department of Research and Development Office, and the office of the Vice President for Research, Extension, and Training for the support. Thanks also to the College of Veterinary Medicine Faculty and staff including the DVM students especially to Dr. Loiuse Jane Dumlao for assisting and for being part of this study. And lastly, to our Almighty God for the divine providence.

References

Ali MZ, Rahman MM, and Sultana S., (2015) Seroprevalence of Mycoplasma gallisepticum antibody by ELISA and serum plate agglutination test of laying chicken. Veterinary World Open Accessed and Peer Reviewed Journal, Volume 8 pp 9-14

Biogal. (2010) Product Information - ImmunoComb® Poultry Mycoplasma (MG-MS-MM) Antibody Test Kit. Accessed online. URL: http://www.biogal.co.il/50pmt103-or-50pmt130?download=34:product-information. pp. 1-4. January 15, 2018.

Biogal. (2012) ImmunoComb® for Mycoplasma. Accessed online. URL: http://glenwoodtechnologies.com/gw/product/ImmunoComb®-for-mycoplasma-mg-ms-mm/. Jan. 15, 2018.

Buim MJ, Mettifogo E., Timenetsky J., Kleven S., Piantino Ferreira AJ. (2009) Epidemiological survey on Mycoplasma gallisepticum and M. synoviae by multiplex PCR in commercial poultry Pesq. Vet. Bras., 29 (2009), pp. 552-556

Butcher GD. (2015) Mycoplasma gallisepticum – A Continuing Problem in Commercial Poultry. Accessed online. URL: http://edis.ifas.ufl.edu/ps034. pp. 1-2. Dec. 18, 2017.

Cortes V., Sevilla-Navarro S., García C., Tudon A., Marín C., Catala-Gregori P. (2021) Seroprevalence and prevalence of Mycoplasma synoviae in laying hens and broiler breeders in Spain. Poultry Science Volume 100 Issue 3

Derksen T., Lampron R., Hauck R., Pitesky M., Gallardo RA., (2018) Biosecurity assessment and seroprevalence of respiratory diseases in backyard poultry flocks located close to and far from commercial premises Avian Disease, Vol. 62, pp. 1-5

Evans JD, Leigh SA, Branton SL, Collier SD, Pharr GT, And SMD Bearson. (2005) Mycoplasma gallisepticum: Current and Developing Means to Control the Avian Pathogen. Poultry Science Association, Inc. pp. 758-759

Feberwee, A, de Vries, TS., Landman, WJM. (2008) Seroprevalence of Mycoplasma synoviae in Dutch commercial poultry farms. Avian Pathology. Volume 37, Issue 6, pp. 629-633

Haghighi-Khoshkhoo P., Akbariazad G., Roohi M., Inanlo J., Masoumi M., and Sami-Yousefi P. (2011) Seroprevalence of Mycoplasma gallisepticum and Mycoplasma synoviae infection in the commercial layer flocks of the Centernorth of Iran, African Journal of Microbiology Research Vol. 5(18), pp. 2834-2837

Haesendonck R, Verlinden M, Devos G, Michiels T, Butaye P, Haesebrouck F, Pasmans F, Martel F (2014) High Seroprevalence of respiratory pathogens in hobby poultry Avian Disease Vol.58, pp. 623-627

Heleili N, Ayachi A, Mamache B, Chelihi A.J. (2012) Seroprevalence of Mycoplasma synoviae and Mycoplasma gallisepticum at Batna Commercial poultry farms in Algeria. Vet. World. 2012;5(12):709–712. doi:10.5455/vetworld.2012.709-712

Hossain KMM, Ali MY, and Haque, MI. (2007) Seroprevalence Of Mycoplasma Gallisepticum Infection In Chicken In The Greater Rajshahi District Of Bangladesh. Bangladesh Society for Veterinary Medicine, 5 (1 & 2): 09–14

Kleven SH. (1998) Mycoplasmas in the Etiology of Multifactorial Respiratory Disease. Department of Avian Medicine, University of Georgia. pp. 1146-1148

Levinsohn S, Hyman H, Perelman D, and Razin S. (1989) The use of a specific DNA probe for detection of Mycoplasmagallisepticum in field outbreaks. Avian Pathology. pp. 535-541.

Ley DH. (2003) Mycoplasma gallisepticum infection. 11th ed. Iowa State University Press. pp. 722-744.

Kapetanov M., Orlic D., Potkonjak D., Velhner M., (2010) Mycoplasma in poultry flocks in the year 2009 compared to the year 2000 and significance of the control measures in Serbia Lucrari Sci. Med. Vet., 43 (2010), pp. 249-253

Ombase DV, Pharande RR, Majee SB, Gandge RS, Thorat VD, Ingle SA, and Moregaonkar SS. (2018) Seroprevalence of Mycoplasma gallisepticum in Broiler Chicken by ELISA. pp. 56-59.

Razin S, Yogev D, and Naot Y. (1998) Molecular biology and pathogenicity of mycoplasmas. Microbiology and Molecular Biology Reviews. Vol 62. pp.1094-1156.

Raviv Z., and Ley, D.H. (2013) Mycoplasma gallisepticum infection D.E. Swayne, J.R. Glisson, L.R. McDougald, L.K. Nolan, D.L. Suarez, N. Venugopal (Eds.), Disease of Poultry (13th edition), Iowa State Press, Ames, pp. 877-893

Seifi S and Shirzad MR. (2012) Seroprevalence and Risk Factors of Mycoplasma gallisepticum infection in Iranian Broiler Breeder Farms. International Journal Animal and Veterinary Advance. pp.45-48.

Sui C., CuiJun H., Yunchao JX., Lunguang K., Yingzuo Y., Xinheng B., Qingmei Z. (2021) Epidemiological investigations and locally determined genotype diversity of mycoplasma synoviaein Central China from 2017 to 2019. Poultry Science Volume 101, Issue 1 pp 1-8

Talkington FD, Kleven SH and Brown J. (1985) An enzyme-linked immunosorbent assay for the detection of antibodies to Mycoplasma gallisepticum in experimentally infected chickens. Avian Diseases. pp. 53-70.

Tan CG, Mahadevan J, Aini I, Sheikh OAR, Abdul RM, and Nadzri S. (2016) Prevalence of Mycoplasma gallisepticum in Commercial Chickens and Free Flying Birds IOSR

Journal of Agriculture and Veterinary Science (IOSR-JAVS), e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 9, Issue 12 Ver. I (Dec. 2016), PP 89-95

Viviana F, Caterina L., Giulia M., Flavio S., Alessandro G., Elena C., Antonietta DF., (2020) Molecular detection and characterization of Mycoplasma gallisepticum and Mycoplasma synoviae strains in backyard poultry in Italy. Poultry Science Volume 99, Issue 2, Pages 719-724

World Organization for Animal Health (OIE) (2012) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 7th edition. Volume 1-2. No. 2.3.5. Office international des epizootics. Paris, France. p. 2.

Scientific Research Publishing

Open Journal of Ecology, 2023, 13, 387-408
https://www.scirp.org/journal/oje
ISSN Online: 2162-1993
ISSN Print: 2162-1985

Quantifying Arborescent Flora Diversity in a Secondary Forest Ecosystem: A Comprehensive Assessment in Nambalan, Mayantoc, Tarlac, Philippines

Baby Jean L. Herbulario[1], Raffy L. Tano[2*], Mileatte P. Omaney[1]

[1]College of Agriculture and Forestry, Tarlac Agricultural University, Camiling, Philippines
[2]College of Agriculture, Forestry and Environmental Sciences, Aklan State University, Banga, Philippines
Email: blherbulario2020@tau.edu.ph, *rltanio@asu.edu.ph, rangerstation18@gmail.com

How to cite this paper: Herbulario, B.J.L., Tano, R.L. and Omaney, M.P. (2023) Quantifying Arborescent Flora Diversity in a Secondary Forest Ecosystem: A Comprehensive Assessment in Nambalan, Mayantoc, Tarlac, Philippines. *Open Journal of Ecology*, **13**, 387-408.
https://doi.org/10.4236/oje.2023.136024

Received: July 24, 2022
Accepted: June 23, 2023
Published: June 26, 2023

Copyright © 2023 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Open Access

Abstract

Forest plays a crucial role in providing essential ecosystem services, including water supply, climate regulation, and biodiversity conservation. This study aimed to assess the current state of the forest in Barangay Nambalan, Mayantoc, Tarlac. The specific objectives were to identify tree species in the area and determine the tree diversity index, with a focus on endemism and conservation status. Two transect lines, each spanning one kilometer, were established and a total of 10 sampling quadrats were surveyed. Ecological parameters (relative frequency, relative density, relative dominance, and importance value index) and diversity indices (Shannon-Weiner index, Simpson's index) were computed using the Paleontological Statistic Software Package for Educational Analysis (PAST 4.03). A comprehensive inventory revealed a total of 756 individuals representing 52 species, 46 genera, and 25 families. Among the recorded species, 10 (17.2%) were endemic and 11 (21.6%) were classified as threatened. The Fabaceae (20.8%), Moraceae (14.6%), and Euphorbiaceae (8.3%) were identified as the most abundant families. The computed diversity indices indicated that Barangay Nambalan retains a diverse forest cover; however, species composition was found to be relatively low. Based on the finding, this study recommends the strict enforcement of protective measures and legislation to mitigate further degradation of the remaining forest in Barangay Nambalan.

Keywords

Forest Diversity, Tree Species, Endemism, Conservation Status, Transect Survey, Diversity Indices

1. Introduction

Arborescent flora, comprising trees, plays a crucial role in forest ecosystems, serving as a key component for resource conservation and management in both rural and urban areas [1]. Forest provide a wide range of valuable services, including water supply and rainfall maintenance, security and nutrition, climate control, and biodiveristy conservation [2], which are essential for meeting immediate human needs. However, the future of global forests and trees is confronted by significant environmental and development challenges on a global scale [3].

The Philippines is recognized as one of the world's megadiverse countries, harboring a rich diversity of life forms in both aquatic and terrestrial ecosystems [4]. Nevertheless, biodiversity is currently under critical threat as human activities contribution to the degradation of natural resources, particularly forests [5]. According to the DENR-FMB [6], the Philippines has witnessed a staggering 70 percent decline in forest cover from 1900 to 2007, with forest area decreasing from 21 million hectares to a mere 6.5 million hectares due to extensive logging activities.

Central Luzon, a significant contribution to the country's economic growth, has witnessed the ongoing degradation of its remaining forestlands and watersheds due to rapid urbanization and development [7]. In Tarlac, located in Central Luzon, a substantial decrease in closed forest area has been observed, declining from 5407 hectares in 2010 to a mere 4.0 percent of the total tree cover by 2020. This decline can be primarily attributed to forest fires and extensive logging activities [6] [8]. Notably, Mayantoc, one of the municipalities in Tarlac, has experienced considerable tree cover loss compared to other regions [9]. Consequently, there exists an urgent imperative to undertake a comprehensive inventory and assessment aimed at identifying the trees species in the area, evaluation their conservation status, and formulating informed plans to safeguard the remaining forest cover. Hence, the main objective of this research was to assess the diversity of tree species in Barangay Nambalan, Mayantoc, Tarlac. Specifically, the study aimed to 1) Identify and Quantify tree species in the area; 2) Determine the tree diversity index and ecological parameters of forest communities; 3) Determine the endemism and conservation status of the tree species present in the area; and 4) To provide a recommendation regarding the present condition of forest in Barangay Nambalan, Mayantoc.

2. Review of Related Literature

2.1. Tree Diversity

An essential aspect of tree diversity research is the assessment of species richness, which refers to the number of tree species present in a given area. High species richness is frequently linked to various ecological functions, such as increased productivity, nutrient cycling, and resistance to disturbances [10]. Chao *et al.* [11] discovered that areas with higher tree species richness exhibited more

significant biomass accumulation and carbon storage in a tropical rainforest.

In addition to species richness, tree diversity studies often examine species composition and evenness. Species composition refers to the specific combination of tree species present in an area, while evenness refers to the relative abundance of different species. Both factors are crucial in understanding community dynamics and ecosystem processes [12]. For instance, a study by Baraloto *et al.* [13] in a neotropical forest revealed that changes in species composition and evenness influenced the functional diversity of tree communities, affecting key ecosystem processes such as nutrient cycling and productivity.

Furthermore, research into the factors that influence tree diversity has provided insights into the mechanisms that shape forest ecosystems. Climate, topography, and soil characteristics have all been shown to influence tree species distribution and diversity [14]. Quesada *et al.* [15] found that tree species composition and diversity varied with soil fertility and hydrological conditions in the Amazon rainforest, highlighting the importance of edaphic factors in shaping forest communities.

Human activities and land-use changes also significantly impact tree diversity. Deforestation, habitat fragmentation, and conversion of forests to agricultural or urban areas have led to the loss of tree species and the homogenization of tree communities [16]. Studies have shown that human disturbances can decrease tree diversity and alter community dynamics, disrupting ecosystem functions [17]. For instance, a study by Laurance *et al.* [18] revealed that selective logging reduced tree species diversity and altered community composition in a tropical forest, affecting carbon storage and nutrient cycling.

Overall, research on tree diversity has emphasized its importance for ecosystem functioning and conservation. Understanding the patterns, drivers, and ecological implications of tree diversity is crucial for effective forest management, conservation strategies, and the sustainable use of forest resources.

2.2. Importance of Biodiversity

Biodiversity, the variety of life on Earth, is critical component of our planet's ecosystems and plays a fundamental role in sustaining the functioning and resilience of natural systems. According to Kanieski *et al.* [19], biodiversity is considered a key indicator of ecosystem well-being and directly reflects the conservation status of a particular area. A diverse and healthy biodiversity provides numerous natural services that are essential for human well-being. People living in rural areas near forests rely on a wide range of forest products for their subsistence, and the income generated from trees and forests is crucial for both rural and urban populations. Biodiversity conservation offers significant benefits in meeting immediate human needs, such as ensuring clean and reliable water resources, protection against floods and storms, and maintaining a stable climate. It also provides social benefits, including education, monitoring, recreation, tourism, and cultural values [20].

In the study conducted by Ludwig and Reynolds [21], several steps were iden-

tified in the investigation of biodiversity, including defining study objectives, delineating the study area, determining sampling methods, collecting and organizing data, measuring species similarity, and characterizing biotic factors. Inventory studies serve as the foundation for biodiversity conservation efforts, as they provide essential information for the sustainable use and protection of biodiversity components. Biodiversity assessments are globally recognized as fundamental activities in achieving sustainable biodiversity conservation [22].

Aureo *et al.* [23] emphasized the importance of understanding biogeographical patterns, species richness variations, and endemic trends in elevationally diverse areas for effective conservation strategies. By comprehending these patterns, conservation efforts can be targeted towards protecting and managing areas of high species richness and endemism.

Ganivet and Blomberg [24] highlighted the need for assessing both tree species diversity and forest structure at local and regional levels to gain insights into the current state of tropical forests and develop effective management strategies for their conservation. While assessments at local scales provide accurate estimates of species richness and forest structure, it is important to extrapolate these findings to regional scales to understand the broader picture of tree species diversity and forest structure.

2.3. Biodiversity Conservation

Biodiversity conservation is a crucial aspect of environmental management and sustainable development. One key aspect of biodiversity conservation is the recognition of the intrinsic value of biodiversity. Biodiversity encompasses a variety of life forms, including genes, species, and ecosystems, and its conservation is essential for maintaining ecological balance and resilience [17]. Biodiversity conservation efforts aim to prevent the loss of species and ecosystems and ensure their long-term survival.

The benefits of biodiversity conservation extend beyond ecological considerations. Biodiversity provides numerous ecosystem services that are vital for human well-being. For instance, intact ecosystems with high biodiversity can enhance water quality, regulate climate, and provide various industries with natural resources such as food, medicines, and materials [25]. Biodiversity conservation also plays a crucial role in supporting livelihoods, particularly for communities that rely on natural resources for their sustenance and income [26].

Biodiversity preservation is critical for ecosystems' continued functioning and resilience. Studies have shown that higher levels of biodiversity contribute to increased ecosystem productivity, stability, and resistance to disturbances [27]. Biodiversity conservation helps protect and restore vital ecological processes, such as pollination, nutrient cycling, and pest regulation, which are essential for maintaining the health and productivity of ecosystems [28].

Various strategies and approaches guide biodiversity conservation efforts. National parks and nature reserves are established to protect critical habitats and species [29]. Additionally, habitat restoration and rewilding initiatives aim to

rehabilitate degraded ecosystems and reintroduce species to their historical ranges [30]. Collaborative efforts involving local communities, government agencies, and non-governmental organizations are crucial for effective biodiversity conservation, as they promote local participation and sustainable resource management [31].

Furthermore, incorporating indigenous knowledge and traditional practices into biodiversity conservation strategies has gained recognition. Indigenous communities often possess valuable knowledge about local ecosystems and have a deep understanding of sustainable resource use [32]. Engaging with indigenous communities can lead to more effective and culturally sensitive conservation practices.

Biodiversity conservation is paramount for maintaining ecological integrity, supporting human well-being, and ensuring sustainable development. It involves preserving species, ecosystems, and ecological processes and requires collaborative efforts and the integration of traditional knowledge. By conserving biodiversity, we can protect the planet's natural heritage and secure a more sustainable future for future generations.

2.4. Conceptual Framework

The INPUT-PROCESS-OUTPUT Model was used by the researcher to provide a general structure and direction for the study. **Figure 1** depicts the study's conceptual framework, in which the input consists of data collection through field inventory, transect and quadrat establishment, and specimen collection and preservation.

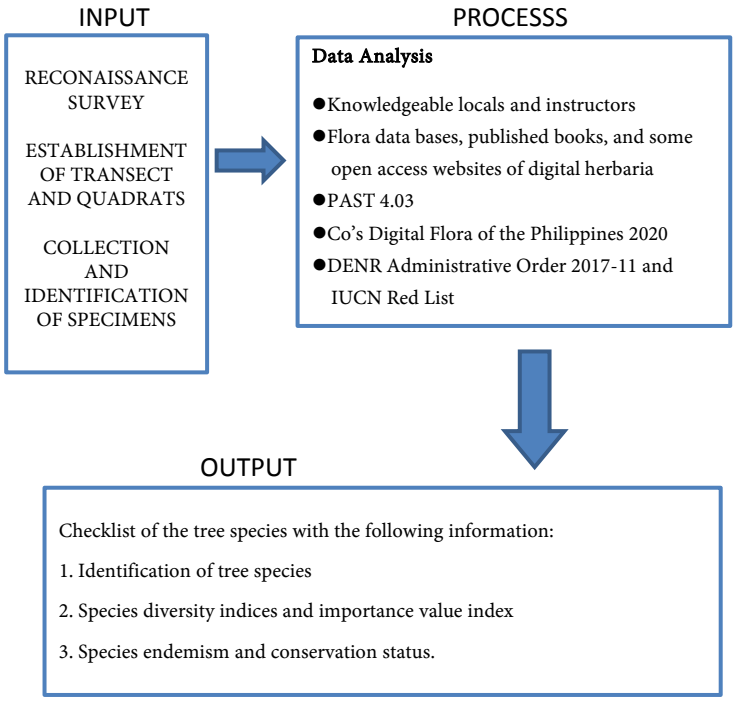


Figure 1. Conceptual framework of the study.

Plant names from family to species level are gathered, the number of individuals of each species, bio-measurements on diameter at breast height (cm), total height (m), and GPS coordinates of all corners of each quadrat. On the other hand, identification of the species was sought with the assistance of knowledgeable locals and instructors, as well as from databases, published books, and some open-access websites of digital herbaria.

For diversity indices, the Shannon Diversity Index formula was used as follows from the studies of Coracero *et al.*, [4]. Species endemicity and conservation status were determined using Co's Digital Flora of the Philippines 2020, D.E.N.R. Administrative Order 2017-11, and the I.U.C.N. Red List. The input and process resulted in identifying tree species present in the area, a tree diversity index, endemism, and the conservation status of tree species in Barangay Nambalan.

3. Methodology

3.1. Locale of the Study

This research was conducted in Barangay, Nambalan, Mayantoc, Tarlac, located in the Northwest part of the Tarlac Province. It is situated at approximately 15.3166 latitude, 120.3166 longitude on the island of Luzon. It is bounded on the west by the Zambales mountain ranges, on the south by the Municipality of San Jose, on the north by the towns of San Clemente and Camiling, and on the east by Santa Ignacia (Figure 2). Nambalan is generally a rough and mountainous area that falls under the climatic Type 1 of the Coronas system of classification, having two pronounced seasons: the dry season from November to May and the wet season during the rest of the year. Its population, as determined by the 2020 Census, was 1570. This represents 4.82% of the total population of Mayantoc as stated [33].

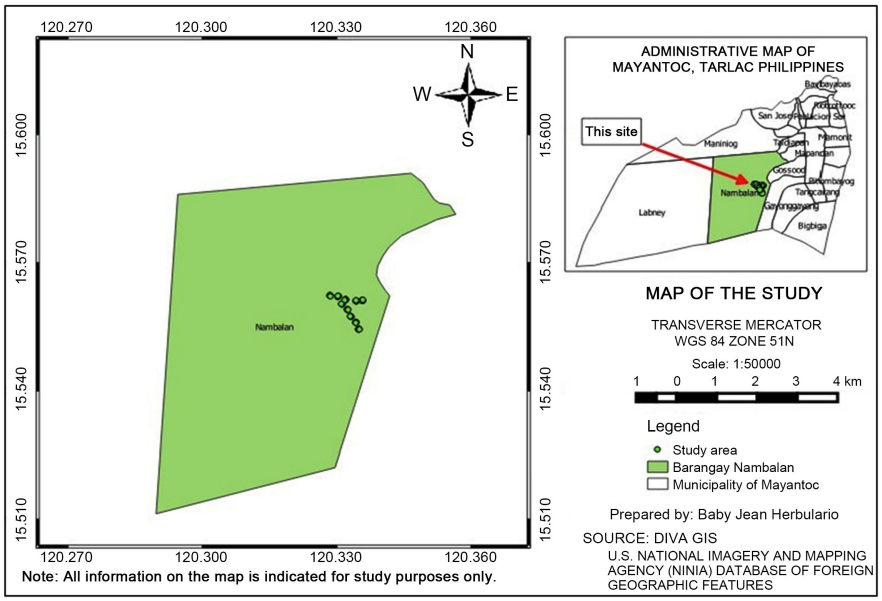


Figure 2. Map showing the location of the study area.

3.2. Data Collection Instruments

The instruments used in data collection were as follows: Procedural activities and formulas were obtained from references, such as previous studies by various researchers, specifically the study conducted [4]. Primary data gathering was through fieldwork activities, mapping and on-site observations, geotagging and tracking using locus map and GeoCam were used to document the activities within the research area.

3.3. Data Collection Procedure

Preliminary procedures were carried out before the conduct of the study. First, the researcher wrote an official letter informing the Barangay captain of the study's purpose and requesting permission to conduct it in the Barangay. This ensured the success of data collection while avoiding suspicion from members of the community. Second, prior to the plot's establishment, a reconnaissance survey was conducted. A mix of quantitative and qualitative analyses was performed. The qualitative component involved identifying the trees encountered per quadrat, while the quantitative component involved computing diversity indices and importance value indices for each species. Field visits, direct observation, and photo documentation were used to characterize the site's vegetation. Secondary data such as area, climatic data, soil, elevation, and other related information were gathered from the Barangay Nambalan Forest Land Use Plan and other online references. The study's mapping activity was created using Quantum GIS (QGIS 2.18) and data generated from the National Imagery and Mapping Agency (NIMA) database, which is accessible online through Diva GIS.

3.4. Transect and Quadrats Establishment

Two (2) transect lines were established with a length of one (1) kilometer each and with ten (10) sampling quadrats having a size of 20 × 20 meters (**Figure 3**). The sampling plots were established in alternating directions on the transect line with 250 meters regular interval.

3.5. Sampling and Data Collection

Biodiversity Assessment

A total of ten (10) 20 × 20-meter sampling plots were established for the identification of trees with at least 5 centimeter in diameter at breast height (DBH). **Figure 4** shows the actual establishment of the plot. Within the 20 m × 20 m sampling plot, tree species with a DBH of at least 5 centimeters were accounted for and measured. DBH, total height, and the number of individuals of each species found in the study area were collected, including GPS coordinates of all corners of each plot. DBH measurements were taken with a diameter tape. The total height of the trees was measured with the aid of an Abney hand level. The crown height and width were measured using estimation, as recommended by Lillo *et al.* [34]. Elevation and trees with flowers and fruits were also observed.

Figure 3. Location of 20 × 20 meters sampling plots.

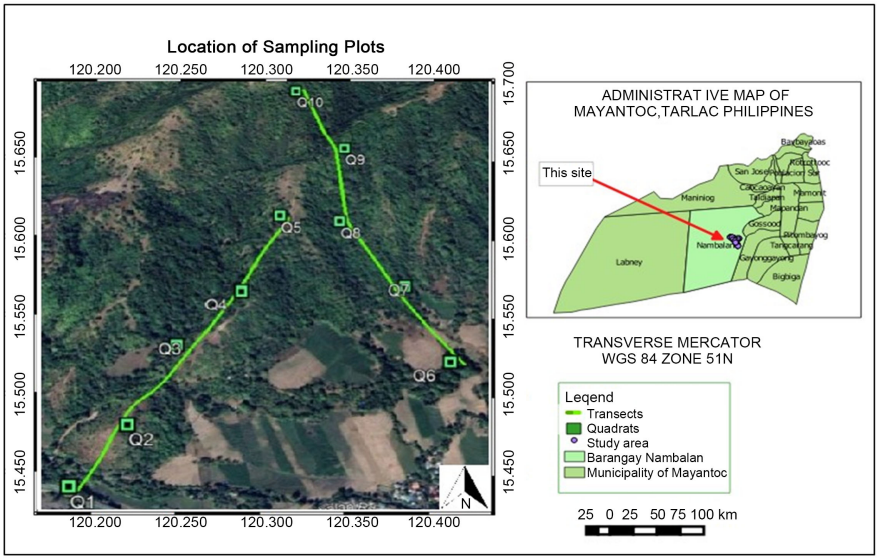


Figure 4. Location of 20 × 20 meters sampling plots.

3.6. Data Analysis

The process of vegetation analysis, including the assessment of tree diversity, computation of diversity indices, identification of tree species, determination of conservation status, and evaluation of species endemism, in this comprehensive discussion. The methods and references cited are based on the research of Coracero and Malabrigo [4], Aureo *et al.* [23], and Lillo *et al.* [34], as well as the use of various botanical resources.

The data collected for the study were utilized for vegetation analysis, specifically focusing on tree species. The evaluation of tree diversity involved the implementation of species abundance measures, such as density, frequency, and dominance. These measures are essential for determining the importance value of each species, which is a standard measurement in forest ecology to establish the rank relationship among different species [34].

The importance value was computed as the cumulative value of relative density, relative frequency, and relative dominance combined into a single metric [23]. This metric provides a comprehensive assessment of the significance of

each species within the ecosystem.

Furthermore, diversity indices were computed using the Paleontological Statistics Software Package for Education and Data Analysis (PAST v.4.03). The interpretation of these diversity indices was based on the Fernando Biodiversity Scale (**Table 1**), as outlined in the study conducted by Coracero and Malabrigo [4]. This scale enables the researchers to understand the level of biodiversity present in the studied area.

To accurately identify tree species, a combination of resources was employed. Local experts, instructors, and knowledgeable locals were consulted to aid in species identification. Additionally, flora databases such as Co's Digital Flora of the Philippines [35] and the International Plant Name Index (IPNI) [36] were utilized. Published books, including Flora Malesiana [37] and Merrill [38], were also consulted. Furthermore, open-access websites of digital herbaria were referenced to access relevant information. The methodologies for species identification and the utilization of these resources were based on the studies conducted by Coracero and Malabrigo [4].

In order to determine the conservation status of each tree species in the Philippines, the researchers relied on the DENR Administrative Order 2017-11. This administrative order provides guidelines and regulations for the conservation of species in the Philippines. Additionally, the conservation status of the species worldwide was determined based on the IUCN Red List, which is maintained by the International Union for Conservation of Nature (IUCN) and provides a comprehensive assessment of the threatened species globally [39].

Furthermore, species endemism was determined using the plant species archive available in the Philippines, namely Co's Digital Flora of the Philippines [35], which is accessible online. This archive provides valuable information on the distribution and endemism of plant species within the Philippines.

The formulas for the computation of various parameters, including importance values, diversity indices, and other relevant metrics, were based on the studies conducted by Coracero and Malabrigo [4]. These studies provided the necessary mathematical frameworks for calculating these parameters accurately and effectively.

$$\text{Dominance} = (0.7854) \times \text{Diameter}^2$$

$$\text{Relative dominane} = \frac{\text{Dominance of a species}}{\text{Sum of the dominance of all species}} \times 100$$

$$\text{Frequency} = \frac{\text{Total number quadrats in which species occured}}{\text{Total number of quadrats studied}}$$

$$\text{Relative Frequency} = \frac{\text{Frequency of a species}}{\text{Sum of frequency of all species}} \times 100$$

$$\text{Density} = \frac{\text{Total number of individuals of species}}{\text{Total number of individual of all species}}$$

$$\text{Relative Density} = \frac{\text{Density of a species}}{\text{Sum of density of all species}} \times 100$$

$$\text{IV} = \text{Relative dominance} + \text{Relative frequency} + \text{Relative density}$$

Table 1. Fernando biodiversity scale.

Relative Values	Shannon Index	Evenness Index
Very high	3.5 and above	0.75 - 100
High	3.0 - 3.49	0.5 - 0.74
Moderate	2.5 - 2.99	0.25 - 0.49
Low	2.0 - 2.49	0.15 - 0.24
Very low	1.9 and below	0.05 - 0.14

4. Results and Discussion

4.1. Tree Species Diversity

The study identified a total of 756 individual trees belonging to 26 families, 46 genera, and 52 species within the two transects. The number of tree individuals per quadrat varied from 35 to 118, with DBH ranging from 5 centimeters to 80 centimeters. Remarkably, *Artocarpus blancoi* and *Syzygium cumini* were the only trees with a DBH of 80 centimeters, suggesting their potential significance in the ecosystem. The majority of species exhibited a DBH of less than 30 cm, indicating that the study area consists of secondary forests in the early stages of vegetation succession [4] [40].

The most abundant families observed were Fabaceae (20%) with ten identified species, Moraceae (13%) with seven identified species, and Euphorbiaceae (9%) with five identified species. This finding suggests that these families play a critical role in influencing the growth and survival of other species in the area [41]. Additionally, Fabaceae species were found to be easily germinating due to their nitrogen-fixing capability [42]. Moraceae and Euphorbiaceae were identified as important food sources for bats and birds, leading to high rates of seed dispersal and successful recolonization [43]. A similar study conducted by Cruz *et al.* [44] in Minalungao National Park in Nueva Ecija reported a high number of Fabaceae species (seven), followed by Moraceae and Euphorbiaceae with six and five species, respectively.

The dominance of Fabaceae, Moraceae, and Euphorbiaceae families highlights their significant role in shaping the vegetation dynamics and supporting the growth and survival of other species. Understanding these ecological relationships is crucial for effective forest management and conservation strategies.

Table 2 shows that quadrat 4 had the highest number of identified species and individual trees, with a total of 33 species and 118 individual trees. In terms of families, quadrat 5 had the highest number, with 23 families. While, quadrat 2, which is dominated by cogon (*Imperata cyclindrica*), was discovered to have the fewest number of individual trees, with a total of 35 individuals, and quadrat 10 was discovered to have the fewest number of families, with 12 families.

These findings demonstrated that variation in species richness was caused by differences in how different species respond to environmental conditions in each plot [45]. Furthermore, dominant species were more likely to maximize their

Table 2. Table summarizing the species composition of the ten sampling quadrats.

Number of Individual		No. of Species	No. of Families
Quadrat	Species		
1	35	19	16
2	37	14	113
3	59	23	21
4	118	33	22
5	94	31	23
6	73	28	20
7	93	32	21
8	100	27	18
9	95	31	19
10	52	22	12
TOTAL	**756**		

ability to capture more resources, resulting in the proliferation of their population over time compared to others [46]. Moreover, the density of species per area influences the development of stem diameter, but other factors such as edaphic and climatic factors may also influence the secondary growth of tree species [47].

Plant species identification was very much dependent on reproductive structures [48]. As a result, in order to perform a more accurate and convenient identification, the reproductive parts of the species must be collected in the field [4]. Furthermore, it could be a source of native species seeds for landscaping and seedling production in the area.

Out of the 52 tree species documented, 13 species were observed with reproductive parts within the sampling sites, as shown in **Figure 5**. These species include *Antidesma ghaesembilla* (Gaertn., Fruct), *Aglaia edulis* (Roxb) Wall., *Buchanania arborescens* (Blume), *Ficus nota* (Blanco) Merr., *Ixora philippinensis* Merr., *Macaranga grandifolia* (Blanco) Merr., *Melanolepis multiglandolusa* (Reinw. Ex Blume) Rchb.f. & Zoll., *Psychotria luzoniensis* (Cham. & Schltdl.) Fern.-Vill, and *Pittosporum pentandrum* (Blanco) Merr. It is noteworthy that all the recorded flowering plant species, like *Pittosporum pentandrum has the ability to suppress and inhibit the growth of Cogon* (*Imperata cylindrica*) *and Talahib* (*Saccharum spontaneum*); *it also produces good soil cover in denuded areas, which is useful for vegetative rehabilitation of degraded areas* [49]. This emphasizes the rich native floral diversity present within the study area and highlights the importance of conserving these native plant species to maintain the ecological balance and support local ecosystems.

In the study area, the presence of reproductive parts in a subset of tree species indicates active reproduction and ecological resilience. Understanding these species' reproductive patterns and characteristics adds to our understanding of their life history strategies and facilitates the development of effective conservation and management plans.

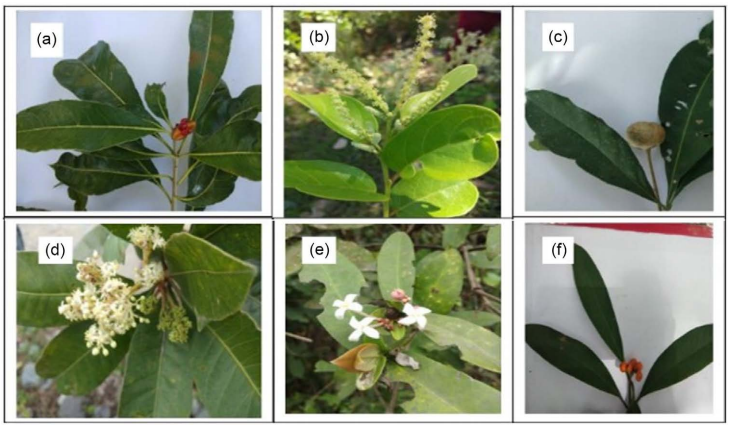


Figure 5. Tree species recorded with reproductive parts within the sampling site. (a) *Pittosporum pentandrum*, (b) *Antidesma ghaesembilla*, (c) *Aglaia edulis*, (d) *Buchanania arborescens*, (e) *Ixora philippinensis*, (f) *Psychotria luzoniensis*.

The predominance of native flowering plant species among the individuals recorded emphasizes the importance of native plant conservation. Native species are frequently better adapted to local environmental conditions and play critical roles in sustaining local biodiversity, ecosystem functions, and overall ecosystem stability.

4.2. Diversity Indices

In the case of Barangay Nambalan, the computed diversity index classifies it as a high diversity area based on the Fernando Biodiversity Scale, with a Shannon diversity index of 3.486 and an evenness index of 0.7098. This indicates a wide variety of species and an even distribution of individuals among them. According to Lillo *et al.* [34], high species diversity in an area contributes to a more stable and productive ecosystem, as diversity is associated with stability, productivity, and trophic structure [50].

Further analysis reveals that six quadrats, specifically two from Transect 1 and four from Transect 2 (**Table 3**), were classified as having high diversity per quadrat. This suggests that these specific areas within Barangay Nambalan contain a considerable number of different species. However, it is important to note that the diversity in Barangay Nambalan is primarily driven by the number of individual species rather than species composition.

This finding aligns with the assertion made by Guiang [51] that a forest community with a higher number of individual species is considered to have high diversity. Additionally, it is known that certain tree species develop specific adaptations that allow them to thrive better than others in a given area [52].

Figure 6 displays the quadrat with the highest number of identified species and individual counts, totaling 33 species and 118 individuals. Prominent species in this quadrat include Alibangbang (*Piliostigma malabaricum* Roxb.), Antipolo (*Artocarpus blancoi* El. Merr), Duhat (*Syzygium cumini* L.), Ipil-ipil (*Leucaena leucocephala*, Lam. De Wit), Kalios (*Streblus asper* Lour., Fl.), Niog-niogan (*Ficus pseudopalma*), Takip-asin (*Macaranga grandifolia*), Tibig (*Ficus nota*), and Yemane (*Gmelina arborea* Roxb).

Table 3. Diversity Indices per quadrats.

Quadrat	Species	Individual	Shannon	Simpson
1	19	35	2.837	0.9355
2	14	37	2.468	0.9028
3	59	23	2.943	0.9371
4	33	118	3.277	0.9563
5	31	94	3.213	0.9529
6	28	73	3.219	0.9559
7	32	93	3.307	0.9573
8	27	100	3.106	0.9472
9	31	95	3.264	0.9567
10	22	52	2.962	0.9423

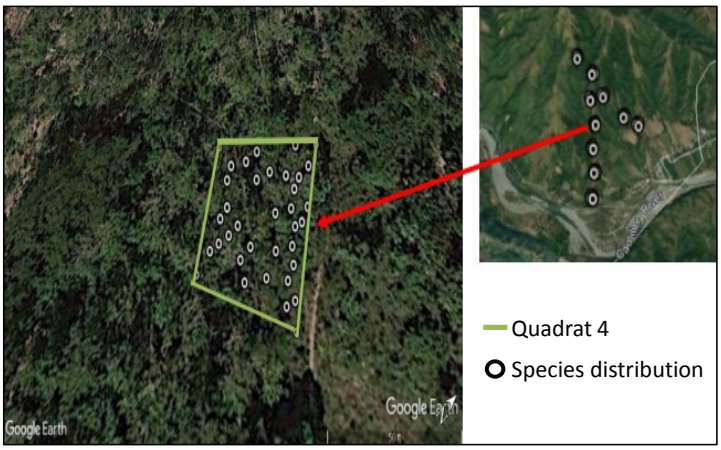


Figure 6. Quadrat with the highest species distributions in the study area.

The diversity observed in this particular plot can be attributed to various factors, including the type of soil, the presence of specific species, and the ground cover. As noted by Cordova *et al.* [53], the role of leaf litter in facilitating plant growth depends on the species. In the case of quadrat 4, which was characterized by a forest floor covered with leaf litter from dominant species, particularly the Fabaceae family, the high diversity observed was expected. Leaf litter promotes soil fertility through microbial activities that are essential for plant growth [53].

Figure 7 shows the plot with the lowest number of identified species and individual species. There were 14 identified species in the area, with 37 individual species. Alibangbang (*Piliostigma malabaricum* (Roxb.) Benth), Akleng parang (*Albizia procera* (Roxb.) Benth), Banato (*Mallotus philippensis* (*Lam.*) *Muell. Arg.*), Binayuyo (*Antidesma ghaesembilla Gaertn*), Hauili (*Ficus septic* (Blanco) Merr.), Lamio (*Dracontomelon edule* (Blanco) Merr.), Mamalis (*Pittosporum pentandrum* (*Blanco*) *Merr*), Molave (*Vitex parviflora* Juss.), Tagpong-gubat (*Psychotria luzoniensis* (*Cham.* & *Schltdl.*) *Fern.-Vill*, *Pitt*) and Takip-asin (*Macaranga grandifolia* (Blanco) Merr.).

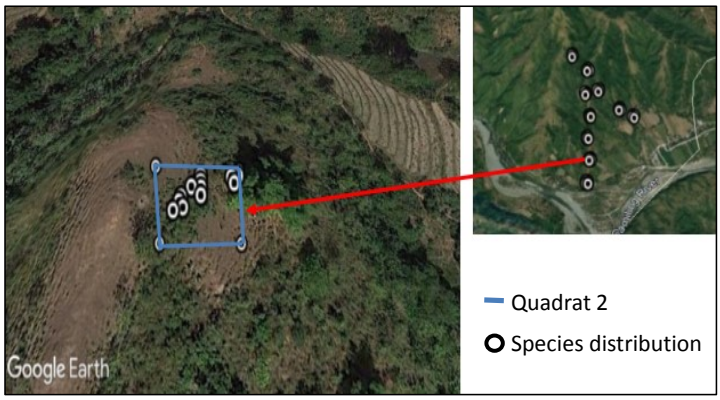


Figure 7. Quadrat with lowest number of identified species.

Based on the observations made during the establishment of the transect, this area was dominated by Cogon grass (*Imperata cylindrica*), which affects the species diversity in the ground cover because cogon commonly over-dominates the vegetation that is prone to burning or fire [53], as well as in areas that are overgrazed and intensively cultivated [54].

Table 4 shows that Transect 1 was more diverse compared to Transect 2, with an average species diversity of 3.46. In terms of species richness, transect 1 had the highest number with 44 species compared to transect 2 with only 41 species, but in terms of number of individual species, transect 2 had the highest number of individuals with 413 individuals, while transect 1 had only 343 individuals.

These findings demonstrated that forest structure varies from lowest to highest elevation in terms of tree diameter, height, and species composition. The type of soil in the area, ground cover, species types, elevation, and climatic conditions all had an impact on these findings. This result confirmed Amoroso *et al.* [55] finding that elevation influences species composition by providing complex environmental gradients such as temperature, rainfall, and relative humidity, as well as different nutrient requirements of the tree species in the area.

4.3. Species Importance Values Index (IVI)

The ten most important species in terms of dominance, frequency, and density were *Piliostigma malabaricum* (18.75), *Artocarpus blancoi* (17.28), *Gmelina arborea* (16.82), *Syzygium cumini* (15.64), *Mangifera indica* (12.64), *Ficus nota* (11.40), and *Psychotria luzoniensis* (Cham). **Figure 8** shows values for *Buchanania arborescens* (11.19), *Ficus septica* (9.74), and *Albizia procera* (9.36). These species are divided into seven (7) families. According to Lillo *et al.* [34], importance value is a quantity that measures the degree of significance of tree species in a given forest community and is derived from three variables, namely density, cover and frequency.

These results indicate that these plants were the most common and the most dominant species in the area [56]. It confirmed that the relative density of each species contributed most to their IV and the floristic composition and vegetation

structure of this area were dependent from these species [53]. In addition, species with high importance value index as shown in **Figure 9**, indicates that the species was well-represented due to a large number of individuals observed compared with other species [57] at the same time it provides an overall estimate of the influence of these species in the community [53].

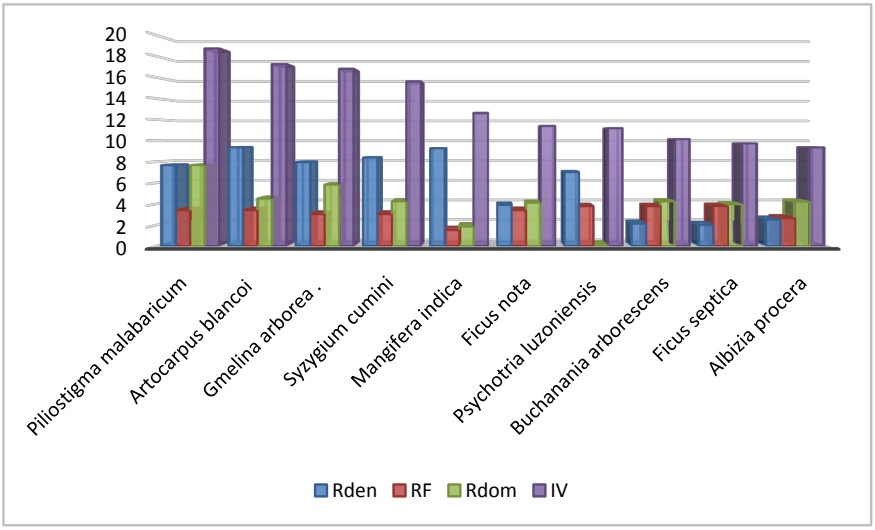


Figure 8. List of species with high Importance Value (IV).

Bauhinia purpurea *Buchanania arborescens*

Syzygium cumini *Ficus nota*

Figure 9. Tree species with high importance value index in the area.

Table 4. Diversity indices values per transect.

Diversity indices	Transect 1	Transect 2	Brgy. Nambalan
Taxa/Species	44	41	46
Individuals	343	413	756
Shannon	3.46	3.413	3.43
Simpson Evenness	0.9617	0.9602	0.9622

4.4. Species Endemism and Conservation Status

As shown in **Table 5** species endemicity assessment revealed nine (9) endemics species comprising 18.8% of the total tree species. It includes *Buchanania arborescens* (Blume), *Semecarpus cuneiformis* Blanco, *Canarium hirsutum* Willd, *Macaranga grandifolia*, *Artocarpus blancoi* (Elm.), *Broussonetia luzonica* Merr, *Ficus nota* (Blanco) Merr., *Ficus pseudopalma* Blanco, FL., *Psychotria luzoniensis* (Cham. & Schltdl.) Fern.-Vill. All endemic species was present both in transect 1 and 2 except for *Canarium hirsutum Willd* which was present only in Transect 2 as shown in **Table 5**.

This result confirmed that endemic species in the area should be given priority in conservation planning because these species are more vulnerable to threats due to their narrow range [58]. In addition, information on the geographic distribution of plant species in the area helps avoid species extinction and, at the same time, it plays an integral basis for the formulation of conservation and management strategies [53].

The conservation assessment was based on the International Union for the Conservation of Nature (2021-3) and DENR Administrative Order No. 2017-11. The assessment result showed that of the 52 species collected, only 11 (21.6%) of the total species were threatened as shown in **Table 6**.

In the IUCN Redlist, seven (7) species were categorized as Vulnerable, namely *Macaranga grandifolia*, *Afzelia rhomboidea*, *Vitex parviflora* Juss., *Sandoricum koetjape* (Burm.f.) Merr., *Artocarpus blancoi* (Elm.) Merr., and *Eucalyptus deglupta* Blume, while *Pterocarpus indicus* was listed as Endangered and *Aglaia edulis* was in the not threatened category (**Table 6**).

While in the DAO 2017-11, or the Updated National List of Threatened Philippine Plants and their Categories, only six (6) species were threatened. Apunan (*Diospyros cauliflora*), Rambutan (*Nephelium lappaceum*), and Narra (*Pterocarpus indicus*) were listed as vulnerable, while Tindalo (*Afzelia rhomboidea*) and Molave (*Vitex parviflora*) were endangered, and *Aglaia edulis* fell into the category of other threatened species, while the remaining five (5) species were not assessed. Among the notable species were Molave (*Vitex parviflora*) and Tindalo (*Afzelia rhomboidea*) (**Table 6**).

This result showed that there were eleven species recorded in this inventory that should be given priority for conservation because the ecological significance of any land formation relies not only on species richness but also on the number of native, endemic, and even threatened species present in the area [59].

Table 5. List of endemic species in the study area.

Family Name	Scientific Name	Common Name	Transect
Anacardiaceae	*Buchanania arborescens*	Balinghasai	1 & 2
	Semecarpus cuneiformis	Kamiring	1 & 2
Burseraceae	*Canarium hirsutum*	Pagsahingin	2
Euphorbiaceae	*Macaranga grandifolia*	Takip-asin	1 & 2
Moraceae	*Artocarpus blancoi*	Antipolo	1 & 2
	Broussonetia luzonica	Himbabao	1 & 2
	Ficus nota	Tibig	1 & 2
	Ficus pseudopalma	Niog-niogan	1 & 2
Rubiaceae	*Psychotria luzoniensis*	Tagpong-gubat	1 & 2

Table 6. Taxonomic list of Threatened species in the study area.

Family Name	Scientific Name	Common Name	DAO 2017-11	IUCN Redlist
Ebenaceae	*Diospyros cauliflora*	Apunan	VU	-
Euphorbiaceae	*Macaranga grandifolia*	Takip-asin	-	VU
Fabaceae	*Afzelia rhomboidea*	Tindalo	EN	VU
	Pterocarpus indicus	Narra	VU	EN
Lamiaceae	*Vitex parviflora Juss.*	Molave	EN	VU
Meliaceae	*Aglaia edulis*	Malasaging	OTS	NT
	Swietenia macrophylla	Mahogany	-	VU
	Sandoricum koetjape	Santol	-	VU
Moraceae	*Artocarpus blancoi*	Antipolo	-	VU
Myrtaceae	*Eucalyptus deglupta*	Bagras	-	VU
Sapindaceae	*Nephelium lappaceum*	Rambutan	VU	-

Legend: **VU**—Vulnerable; **EN**—Endangered; **OST**—Other Threatened Species; **NT**—Not assessed.

5. Conclusion and Recommendation

Based on the results, Barangay Nambalan is dominated by families Fabaceae, Moraceae, and Euphorbiaceae. There were 10 (17.2%) endemic species and 11 (21.6%) threatened species recorded in the area out of 52 species. From low to high elevation, the forest structure varies in terms of tree diameter, height, and species composition. These findings were influenced by the type of soil in the area, ground cover, types of species, elevation, and climatic conditions. The survey documented 52 species belong to 26 families and 46 genera, with 756 individuals. The most abundant, most frequent, and most important species found in the area was *Piliostigma malabaricum*, with 59 individual species, occurring in 9 out of 10 quadrats. In terms of species diversity, Barangay Nambalan is still diverse in terms of individual species but low in terms of species composition,

with a Shannon-Weiner Index of 3.43 and Simpson Evenness of 0.7068.

The researcher recommends that laws governing timber cutting as stated in Section 77 of Presidential Decree (PD) 705 should be strictly enforced in order to reduce human activity in the area, such as illegal logging. Barangay local government units should craft a policy regarding land use plans to allocate their resources and protect the area's diversity. Also, the Barangay should conduct tree planting activities every year to conserve and protect their remaining resources in denuded forestland. Additionally, educate local residents, as well as the tourists who visit the area about the importance of native and endemic species through information education and communication publications. Furthermore, studies in other parts of the forest would be preferable to better assess its diversity so that the initial information gathered can be supplemented by additional survey data to create a good reference document for conservation and management in the area.

Acknowledgements

The authors sincerely thank the Barangay Local Government Unit (B.L.G.U.) and Hon. Arnold Reyes for granting permission to conduct the Diversity Assessment of Arborescent Flora. Special thanks are also extended to For. Julius G. Pascua, For. Mark Anthony L. Bagsit, and For. Christian Llait for their valuable assistance and suggestions throughout the research process. Finally, the authors would like to acknowledge the crucial support provided by the TAU CAF-Forestry Department, without which this publication would not have been possible.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Raji, I.A. and Babalola, F.D. (2018) Assessment of Tree Diversity and Abundance in University of Ilorin Campus: Towards Conservation. *Proceedings of 6th NSCBBiodiversity Conference*, Uniuyo, 2018, 443-450.

[2] Coracero, E., Urriza, H. and Foster, P.G. (2021) Species Composition and Diversity: Baseline for Tree Conservation at Laguna State Polytechnic University, San Pablo, Philippines. *International Research Journal of Advanced Science*, **2**, 10-16.

[3] Holmgren, J. and Persson, A. (2003) The Future of the World's Forests: Ideas vs. Ideals. *Ambio*, **32**, 541-548.

[4] Coracero, L.M. and Malabrigo, P.L. (2020) The Philippines as a Megadiverse Country: Perspectives on Conservation and Sustainable Development. *Philippine Journal of Science*, **149**, 727-742.

[5] Cabansag, J.B.C. (2016) Biodiversity Conservation in the Philippines: Challenges, Prospects and Policy Directions. *Philippine Science Letters*, **9**, 1-9.

[6] DENR-FMB (2018) Philippine Forestry Statistics 2017-2018. DENR-FMB. https://forestry.denr.gov.ph/

[7] NEDA (2017) Central Luzon Regional Development Plan 2017-2022. National Economic and Development Authority. https://www.neda3.net.ph/

[8] DENR-FMB (2015) Philippine Forestry Statistics 2014-2015. DENR-FMB. https://www.forestry.denr.gov.ph/

[9] Global Forest Watch (2022) Tree Cover Loss by Country. Global Forest Watch. https://www.globalforestwatch.org/

[10] Huston, M.A. (2014) Disturbance, Productivity and Species Diversity: Empiricism versus Logic in Ecological Theory. *Ecology*, **95**, 2382-2396. https://doi.org/10.1890/13-1397.1

[11] Chao, A., Chazdon, R.L., Colwell, R.K. and Shen, T. (2005) A New Statistical Approach for Assessing Similarity of Species Composition with Incidence and Abundance Data. *Ecology Letters*, **8**, 148-159. https://doi.org/10.1111/j.1461-0248.2004.00707.x

[12] Magurran, A.E. (2004) Measuring Biological Diversity. Blackwell Publishing, Oxford, 256.

[13] Baraloto, C., Paine, C.E.T., Poorter, L., Beauchene, J., Bonal, D., Domenach, A.M., Herault, B., Patiño, S., Roggy, J.C. and Chave, J. (2012) Decoupled Leaf and Stem Economics in Rain Forest Trees. *Ecology Letters*, **15**, 887-895.

[14] Garcia-Guzman, G., Meave, J.A., Moreno, C.E., Martinez-Ramos, M. and Gallardo-Cruz, J.A. (2017) Determinants of Tree Species Distribution and Diversity at Regional Scales in a Mexican Tropical Dry Forest. *PLOS ONE*, **12**, e0184410.

[15] Quesada, C.A., Lloyd, J., Schwarz, M., Patiño, S., Baker, T.R., Czimczik, C. and Gloor, M. (2011) Variations in Chemical and Physical Properties of Amazon Forest Soils in Relation to Their Genesis. *Biogeosciences*, **8**, 732-755.

[16] Gibson, L., Lee, T.M., Koh, L.P., Brook, B.W., Gardner, T.A., Barloe, J. and Sodhi, N.S. (2011) Primary Forests Are Irreplaceable for Sustaining Tropical Biodiversity. *Nature*, **478**, 378-381. https://doi.org/10.1038/nature10425

[17] Diaz, S., Lavorel, S., Mclntyre, S., Falczuk, V., Casanoves, F., Milchunas, D.G. and Quetier, F. (2007) Plant Trait Responses to Grazing-A Global Synthesis. *Global Change Biology*, **13**, 313-341. https://doi.org/10.1111/j.1365-2486.2006.01288.x

[18] Laurence, W.F., Andrade, A., Magrach, A., Camargo, J.L., Campbell, M., Fearnside, P.M. and Lovejoy, T.E. (2012) Apparent Environmental Synergism Drives the Dynamics of Amazonian Forest Fragments. *Ecology*, **93**, 1764-1769.

[19] Kanieski, R., Longhi, S. and Soares, P. (2017) Methods for Biodiversity Assessment: Case Study in an Area of Atlantic Forest in Southern Brazil. Selected Studies in Biodiversity. InTech. https://doi.org/10.5772/intechopen.71824

[20] MEA (Millenium Ecosystem Assessment) (2005) Ecosystems and Human Well-Being: Synthesis. Island Press, Washington DC.

[21] Ludwig, J.A. and Reynolds, J.F. (1988) Statistical Ecology: A Primer on Methods and Computing. Wiley, New York, 337.

[22] Margules, C.R. and Pressey, R.L. (2000) Systematic Conservation Planning. *Nature*, **405**, 243-253. https://doi.org/10.1038/35012251

[23] Aureo, W.A., Jr Reyes, T.D., Mutia, F.C.U., Tandang, D.N. and Jose, R.P. (2021) Floristic Composition and Community Structure along the Elevation Gradient of Balinsasayao Twin Lakes Natural Parkin Nergros Oriental, Philippines. *One Ecosystem*, **6**, e56536. https://doi.org/10.3897/oneeco.5.e56536

[24] Ganivet, E. and Bloomberg, M. (2019) Towards Rapid Assessments of Tree Species Diversity and Structure in Fragmented Tropical Forests: A Review of Perspectives

Offered by Remotely Sensed and Field-Based Data. *Forest Ecology and Management*, **432**, 40-53. https://doi.org/10.3897/oneeco.5.e56536

[25] Cardinale, B.J., Duffy, J.E., Gonzales, A., Hooper, D.U., Perrings, C., Venail, P., *et al.* (2012) Biodiversity Loss and Its Impact on Humanity. *Nature*, **486**, 59-67. https://doi.org/10.1038/nature11148

[26] Adams, W.M., Aveling, R., Brockington, D., Dickson, B., Elliott, J., Hutton, J., Roe, D., Vira, B. and Wolmer, W. (2004) Biodiversity Conservation and the Eradication of Poverty. *Science*, **306**, 1146-1149. https://doi.org/10.1126/science.1097920

[27] Timan, D., Isbell, F. and Cowles, J.M. (2014) Biodiversity and Ecosystem Functioning. *Annual Review of Ecology, Evolution and Systematics*, **45**, 471-493. https://doi.org/10.1146/annurev-ecolsys-120213-091917

[28] Isbell, F., Craven, D., Connolly, J., Loreau, M., Schmid, B., Beierkuhnlein, C. and Vab Ruijven, J. (2013) Biodiversity Increases the Resistance of Ecosystem Productivity to Climate Extremes. *Nature*, **494**, 249-252.

[29] Joppa, L.N., O'Connor, B., Visconti, P., Smith, C., Geldmann, J., Hoffmann, M., *et al.* (2016) Filling in Biodiversity Threat Gaps. *Science*, **352**, 416-418. https://doi.org/10.1126/science.aaf3565

[30] Seddon, P.J., Griffiths, C.J., Soorae, P.S. and Armstrong, D.P. (2014) Reversing Defaunation: Restoring Species in a Changing World. *Science*, **345**, 406-412. https://doi.org/10.1126/science.1251818

[31] Oldekop, J.A., Holmes, G., Harris, W.E. and Evans, K.L. (2019) A Global Assessment of the Social and Conservation Outcomes of Protected Areas. *Nature Sustainability*, **2**, 452-459.

[32] Gadgil, M., Berkes, F. and Folke, C. (1993) Indigenous Knowledge for Biodiversity Conservation. *Ambio*, **22**, 151-156.

[33] PhilAtlas (2022) Nambalan Municipality of Mayantoc, Province of Aklan. PhilAtlas. https://www.philatlas.com/luzon/r03/tarlac/mayantoc/nambalan.html

[34] Lillo, E.P., Fernando, E.S. and Lillo, M.R. (2021) Plant Diversity and Structure of Forest Habitat Types on Dinagat Island, Philippines. *Journal of Asia-Pacific Biodiversity*, **12**, 83-105. https://www.ukdr.uplb.edu.ph/journal-articles/770 https://doi.org/10.1016/j.japb.2018.07.003

[35] Co's Digital Flora of the Philippines (2020) https://www.philippineplants.org/

[36] International Plant Names Index (IPNI) (2016) https://www.ipni.org/

[37] (2019) Flora Malesiana. Naturalis Biodiversity Center, Leiden.

[38] Merrill, E.D. (1922) An Enumeration of Philippine Flowering Plants. Bureau of Printing, Manila. https://doi.org/10.5962/bhl.title.49412

[39] International Union for Conservation of Nature (IUCN) (2021) The IUCN Red List of Threatened Species. IUCN. https://www.iucnredlist.org/

[40] Galindon, J., Pasion, B., Tongco, M.D., Fidelino, J., Duya, M.R. and Ong, P. (2017) Plant Diversity Patterns in Remnant Forests and Exotic Tree Species-Based Reforestation in Active Limestones Quarries in the Luzon and Mindanao Biogeographic Sub-regions in the Philippines. *Ecological Research*, **33**, 63-72. https://doi.org/10.1007/s11284-017-1533-5

[41] Van der Ent, A., Repin, R., Koller, B., Delang, C.O. and Sterck, F.J. (2013) Tree Communities of Ultramafic Forests in Sabah (Malaysia). *Plant Ecology and Evolution*, **146**, 292-305.

[42] Wang, W.Q., Wang, Y.J. and Zhang, J.L. (2010) Germination Characteristics of 29 Leguminous Plants. *Acta Prataculture Sinica*, **19**, 230-238.

[43] Lomascolo, S.B., Schor, A. and Fernandez, M. (2010) Birds and Bats as Seed Dispersers of Fleshy-Fruited Plants in a Natural Reforestation area in South Brazil. *Brazilian Journal of Biology*, **70**, 581-587.

[44] Cruz, L.E., Ibanez, N.N., Sajise, A.J. and Vinuya, F.M. (2018) Floristic Composition and Diversity of Minalungao National Park, Nueva Ecija, Philippines. *Asia Journal of Biodiversity*, **9**, 159-177.

[45] Krebs, C.J. (1999) Ecological Methodology. Benjamin-Cummings, San Francisco.

[46] Dela Cruz, E.G., GojoCruz, P.H.N., David, E.S., Abella, E.A. and Cruz, K.G.J. (2018) First Report of Tree Species of Minalugao National Park, Nueva Ecija, Philippines. *Journal of Biodiversity and Environmental Sciences* (*KBES*), **13**, 56-62. http://www.innspub.net

[47] Raga-as, M.L., Saladar, R.L., Calaguio, J.C., Tano, R.L. and Unlayao, G.M.C. (2022) Assessment of Physical Characteristics of Bugang River Watershed in Pandan, Antique, Philippines. *Open Journal of Ecology*, **12**, 360-376. https://doi.org/10.4236/oje.2022.126021

[48] Umali, A.A., Malabrigo, P.L. and Replan, E.L. (2018) Floral Diversity and Habitat Assessment at Mt. Malarayat Brgy. Malitlit, Lipa City, Batangas. *Ecosystems & Development Journal*, **8**, 3-14.

[49] Tano, R.T. (2023) Effect of Pre-Sowing Treatments on Mamalis (*Pittosporum pentandrum* Blanco Merr.) Seeds Germination under Nursery Condition. *Open Journal of Ecology*, **13**, 106-118. https://doi.org/10.4236/oje.2023.132009

[50] Mclntosh, R.P. (1967) The Species Diversity Gradient and the Distribution of Higher Taxa. *Ecology*, **48**, 648-656.

[51] Guiang, E.S. (2001) Efficacy of Removing Natural Forest from Timber Production as a Strategy for Conserving Forests in the Philippines. In: Durst, P., Waggener, T., Enters, T. and Cheng, T., Eds., *Forests Out of Bounds*: *Impacts and of Logging Ban in Natural Forests in Asia-Pacific*, FAO.

[52] Baker, A.J.M., MacGrath, S.P., Reeves, R.D. and Smith, J.A.C. (2000) Metal Hyperaccumulator Plants: A Review of the Ecology and Physiology of a Biological Resource for Phytoremediation of Metal-Polluted Soils. In: Terry, N. and Bañuelos, G.S., Eds., *Phytoremediation of Contaminated Soil and Water*, Taylor and Francis, Abingdon-on-Thames.

[53] Cordova, L.V., Malabrigo, P.L., Tiburan, C.L., DeGuia, A.O., Labatos, B.V., Balatibat, J.B., Umali, A.A., Pantau, K.V., Eduarte, G.T., Tobias, A.B., Evasco, J.J. and Divina, A.N. (2019) Assessment Study for New Clark City. Project No. 50, Pro-Seeds Development Association Inc., Los Baños.

[54] Wibowo, A.M., Suharti, A.P.S., Sagala, H., Hibani, H. and Van Noordwijk, M. (1997) Fire Management on Imperata Grasslands as Part of Agroforestry Development in Indonesia. *Agroforestry Systems*, **36**, 203-217. https://doi.org/10.1007/BF00142874

[55] Amoroso, V.B., Laraga, S.H. and Calzada, B.V. (2011) Diversity and Assessment of Plants in Mt. Kitanglad Range, Natural Park, Bukidnon, Southern Philippines. *Garden's Bulletin Singapore*, **63**, 219-236.

[56] Alberto, A.P. and Cabutaje, A.P. (2018) Plant Diversity Assessment in The Ecotone Ecosystem of Sitio Bulac, Barangay General Luna, Carranglan, Nueva, Ecija, Philippines. *Asia Journal of Biodiversity*, **9**, 37-51. https://doi.org/10.7828/ajob.v9i1.1233

[57] Hodabalo, P., Kperkouma, W., Thomas, T., Balinga, M., Bessieke, Z., Dourma, M., Batawila, K. and Akpagaca, K. (2013) Woody Species Diversity and IVI in Dense Dry Forests in Abdoulaye Wildlife Reserve (Togo, West Africa). *International*

Journal of Biodiversity and Conservation, **5**, 358-366.

[58] Malabrigo, P.L.Jr., Tobias, A.B. and Boncodin, J.C. (2018) FloristicComposition, Vegetation Structure, and Diversity Pattern of Mt. Calavite Wildlife Sanctuary: Basis for Management and Conservation Planning. *Ecosystems & Development Journal*, **8**, 3-27.

[59] De Castro, M., Carandang, J.S. and Agoo, E. (2020) Floristic study of an Ultramafic Formation in Sitio Magarwak, Sta. Lourdes, Puerto Princesa City, Palawan Island, Philippines. *Biodiversitas Journal of Biological Diversity*, **21**, 3769-3779. https://doi.org/10.13057/biodiv/d210844

Journal of Applied Pharmaceutical Science Vol. 12(07), pp 184-189, July, 2022
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2022.120718
ISSN 2231-3354

Regulation of the immune system by administering lactic acid bacteria to suppress the effects of aflatoxin B1 in mice (*Mus musculus*)

Dahliatul Qosimah[1*], Sri Murwani1, Indah Amalia Amri[1], Agri Kaltaria Anisa[2], Ma. Asuncion Guiang Beltran[3]

[1]Laboratory of Microbiology and Immunology, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, Indonesia.
[2]Laboratory of Pharmacology, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, Indonesia.
[3]College of Veterinary Medicine, Tarlac Agricultural University, Camiling, Tarlac, Philippines.

ARTICLE INFO

Received on: 15/12/2021
Accepted on: 10/04/2022
Available Online: 05/07/2022

Key words:
Lactic acid bacteria, toxin, fungi, cellular immune, humoral immune.

ABSTRACT

Aflatoxin B1 (AFB1), which is a toxic compound produced by the filamentous fungus *Aspergillus* sp., is highly carcinogenic, damages vital organs, and may cause death. Prevention of aflatoxin poisoning through proper food storage and physical treatment is an added cost, thus there is a need to identify alternative methods including treatment with probiotic bacteria. We evaluated the effect of *Lactobacillus bulgaricus* on activating immune cells in mice exposed to Aflatoxin B1. The study used a post-test control design consisting of five treatment groups including a negative control, positive control, and T1, T2, and T3 groups treated with lactic acid bacteria at doses of 105 colony forming unit (CFU)/ml, 107 CFU/ml, and 109 CFU/ml, respectively, administered on days 7–28 and AFB1 at a dose of 0.2 mg/kg bw orally on days 15–28. The relative number of lymphocytes consisting of CD11c+transforming growth factor-beta (TGF-β)+, CD4+CD8+, and B220+IgG+, was measured using flow cytometry. The data were analyzed using a one-way analysis of variance test. The results indicated that *L. bulgaricus* bacteria increased the relative number of CD11c+TGF-β+, B220+IgG+, and CD4+CD8+ cells in mice exposed to the mycotoxin. *Lactobacillus bulgaricus* may function as an immunostimulator against mycotoxins by inducing the humoral and cellular immune response.

INTRODUCTION

Mycotoxins, such as aflatoxin B1 (AFB1), are secondary metabolites produced from filamentous fungi that are toxic, carcinogenic, and immunosuppressive to animals and humans. The estimated world production of feed contaminated with fungus is approximately 5%–10% (Oswald *et al*., 2005; Qian *et al*., 2012; Tomkova *et al*., 2001). Aflatoxin reportedly caused up to 100,000 turkey deaths following the consumption of contaminated peanut mushrooms. Broiler feed contaminated with a mycotoxin mixture (3.5 mg/kg diet as 79% of AFB1, 16% AFG1, 4% AFG2, and 1% AFB2) can lead to weight loss and inflammation of the liver and kidneys. Aflatoxin B1 is a potent agent that causes immunosuppression in pigs at a dose of 140–280 µg/kg of feed by inhibiting DNA synthesis and immune cells, such as lymphocytes, but does not affect the humoral immune response (Perczak *et al*., 2018; Pierron *et al*., 2016).

Aflatoxin B1 suppresses the cellular immune system, in particular T lymphocytes, because of decreased complement production by the liver, phagocytosis by macrophages, and neutrophil activity (Perdigon *et al*., 2001). T lymphocytes affected by the toxin, as well as other lymphoid cells, such as cytotoxic T cells and natural killer cells, can promote tumor cell function directly or indirectly. The cellular components of the immune system produce cytokines for protection against tumor progression; however, cytokines may also play a role in the inflammation mechanism that causes damage to various organs (Ibrahim, 2013).

Methods such as heating, chemical treatment, or radiation can destroy and eliminate mycotoxin (Zain, 2011);

*Corresponding Author
Dahliatul Qosimah, Laboratory of Microbiology and Immunology, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, Indonesia.
E-mail: dahlia_qosimah @ ub.ac.id; dahliatulqosimah @ gmail.com

© 2022 Dahliatul Qosimah *et al*. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/).

however, the cost is prohibitive and it may impair the nutritional value of the feed. The decline in fungal growth may increase during feed production or storage (Munoz *et al*., 2010). Lactic acid bacteria (LAB) may exhibit antifungal activity, especially *Lactobacillus* sp (Sadiq *et al*., 2019). *Lactobacillus pentosus* and *Lactobacillus brevis* bacterial strains at a concentration of 3.5 × 108 colony forming unit (CFU)/ml can bind and release aflatoxin B1 by 17.4% and 34.7%, respectively, in liquid media as measured by ELISA (Hamidi *et al*., 2013).

Microorganisms, such as *Saccharomyces cerevisiae* and LAB, may be used as biopreservatives in feeds, so it is possible to extend the shelf-life and increase food safety with microflora supplementation. Antimicrobial products of microorganisms also have potential as probiotics and may improve health (Tran *et al*., 2020); however, there is limited data demonstrating the immunostimulating effects of LAB. Therefore, we evaluated the effect of LAB on the cellular and humoral immunity profile of mice.

MATERIAL AND METHODS

Preparation of animal

Male mice (*Mus musculus*), strain Balb/c aged 8–12 weeks (n = 25), with a body weight of 25–30 g, were obtained from Brawijaya University's bioscience laboratory and divided into five groups (five mice/group). The treatment groups consisted of a positive control (mice induced with AFB1 0.2 mg/kg bw on days 15–28), negative control (healthy mice), and treatment groups, T1, T2, and T3 in which mice were administered 1 × 105 CFU/ml, 1 × 107 CFU/ml, and 1 × 109 CFU/ml of LAB on days 7–28, respectively.

Bacterial suspension preparation

Lactobacillus bulgaricus (LAB) was obtained from the Microbiology Laboratory of the Faculty of Medicine, Universitas Brawijaya, and confirmed by biochemical tests and Gram staining. The bacteria were grown on de Man, Rogosa, and Sharpe (MRS) agar media at 37°C for 24 hours. A bacterial suspension was prepared using MRS broth media and the bacterial concentrations were measured using a spectrophotometer. The bacteria were diluted with phosphate buffer saline (PBS) for the experiments.

Aflatoxin B1 preparation

Aflatoxin B1 (Sigma Company catalog: A6636®) is potent with respect to acute toxicity, mutagenicity, and carcinogenicity, and one vial of AFB1 contained 5 mg of powder. AFB1 (0.2 mg/kg BW) was diluted in 1 ml PBS, pH 7.2 (Qian *et al*., 2012).

Flow cytometry

The mice were sacrificed on day 29 by cervical dislocation. Spleens were harvested, placed into a petri dish containing sterile PBS, and crushed with the base of a syringe. 10 ml homogenates of the sample were prepared in a volume of 10 ml, centrifuged, and the pellets were resuspended in 1 ml PBS by pipetting (Ardiana and Rifa'i, 2015).

Then, 100 µl of the suspension was placed into a microtube; 500 µl of PBS was added; and the mixture was centrifuged at 2,500 rpm for 5 minutes at 4°C. Then, 50 µl of PE/Cy5 conjugated rat anti-mouse Cd11c, PECy5 conjugated rat anti-mouse transforming growth factor-beta (TGF-β), fluorescein isothiocyanate (FITC) conjugated rat anti-mouse CD4, PE conjugated rat anti-mouse CD8, FITC conjugated rat anti-mouse B220, and PECy5 conjugated rat anti-mouse IgG (Biolegend®, San Diego) were added to the cells and incubated for 20 minutes in 4°C (8). Afterward, 50 µl of cytofix (BD Biosciences Pharmingen) was added and incubated for 20 minutes in 4°C; wash perm solution (BioLegend®, USA) was added; and the mixture was centrifuged at 2,500 rpm at 10°C for 10 minutes. The cells were analyzed by flow cytometry (BD FACSCalibur, USA) using BD Cellquest ProTM software.

Statistical analysis

The data are presented as the relative number of immune cells (CD11c+TGFβ+, CD4+CD8+, and B220+IgG+). Data were analyzed statistically using one-way analysis of variance (ANOVA) with an error level of $\alpha = 0.05$, followed by Tukey's test.

RESULTS

$CD11c^+TGF\text{-}\beta^+$ cells

The results indicated that supplementation of the mice with LAB induced by AFB1 increased the relative number of CD11c+ cells that produce TGFβ+ (Fig. 1). In the positive control group, the relative number of CD11c+ expressing molecule TGF-β+ (1.75%) was different, but no significance was observed when compared with the negative control (0.91%). All treatment groups (T1, T2, and T3) administered LAB at a 105–109 CFU/ml concentration showed an increase in the relative number of CD11c+TGF-β+ cells by 2.05% 3.14%, and 3.06%, respectively, when compared to the negative and positive controls, as shown in Figure 1.

$CD4^+CD8^+$ cells

The results showed that the supplementation with LAB in mice induced with AFB1 increased the relative number of CD4+CD8+ cells in all treatment groups, but the amount did not significantly differ and was similar to that of the negative control, as shown in Figure 2. The negative control was higher when compared with the positive control.

$B220^+IgG^+$ cells

The results showed significant differences in the relative number of B220+-expressing IgG+ cells in all the treatment groups following supplementation with LAB after induction with AFB1. The negative control group (24.56%) was significantly different from the positive control group (5.76%), which showed a relatively higher number of B220+IgG+ cells compared with the positive control. There was an increase in the relative number in all treatment groups (7.44%, 10.26%, and 7.67% for T1, T2, and T3, respectively), as shown in Figure 3.

DISCUSSION

Effect of LAB on the relative number of $CD11c^+TGF\text{-}\beta^+$ cells

The results indicated that the relative number of CD11c+ cells that expressed TGF-β was different, but there was no significant between the treatment groups; however, a 107 CFU/ml concentration of LAB increased the average of CD11c+TGF-β+

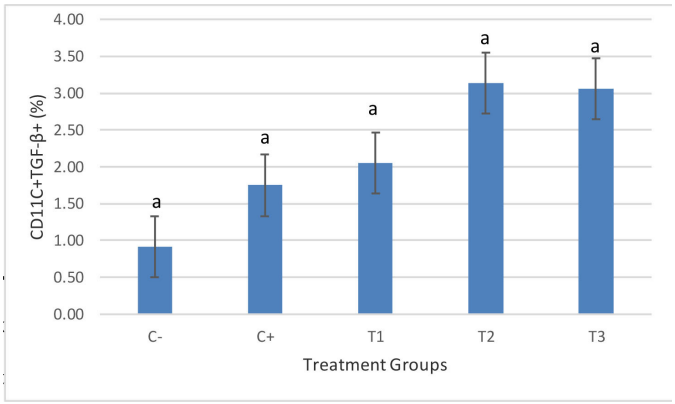


Figure 1. Flow cytometry analysis shows that giving LAB showed an increase in the relative number of CD11c+TGF-β+ cells ($p < 0.05$), but was not significantly different between treatments. There was an increase in the number when compared with the positive control. The highest average increase was seen in the T2 treatment. The treatment groups are: C- (healthy mice), C+ (mice induced with AFB1), and treatment groups, T1, T2, and T3 in which mice were administered with AFB1 and 1 × 105 CFU/ml, 1 × 107 CFU/ml, and 1 × 109 CFU/ml of LAB on days 7–28, respectively.

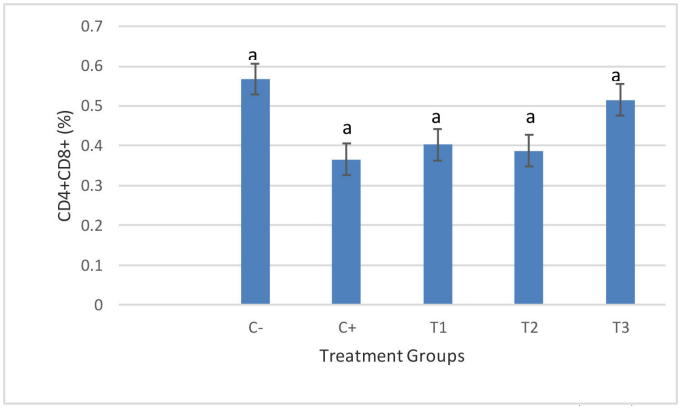


Figure 2. Treatment with LAB showed an increase in the relative number of CD4+CD8+ cells for all treatments, which did not differ significantly ($p < 0.05$). The treatment groups are: C- (healthy mice), C+ (mice induced with AFB1), and treatment groups, T1, T2, and T3 in which mice were administered with AFB1 and 1 × 105 CFU/ml, 1 × 107 CFU/ml, and 1 × 109 CFU/ml of LAB on days 7–28, respectively.

cells in all treatments. Mycotoxin exposure decreased the relative amount of CD11c+TGF-β+ cells. Mycotoxin AF1 altered or decreased anti-inflammatory cytokine synthesis by inhibiting macrophage or T cell activation. In fact, mycotoxin inhibits the synthesis and proliferation of T cells, which prevents macrophage cells from producing anti-inflammatory cytokines. Mycotoxin is cytotoxic to lymphocytes by interfering with lymphocyte receptors or lymphocyte function (Tran *et al*., 2020). A study by Murugesan *et al*. (2015) revealed that mycotoxin was not immunogenic. It could not induce an immune response to pathogens, but interfered with mitogen-activated protein kinase signaling to modulate cell growth, apoptosis, or the immune response. This could expose an individual to a high risk of infection.

Dendritic cells (DC) and CD11c markers are antigen-presenting cells (APCs) that are regulated specifically and nonspecifically by immune cells found in the lamina propria of the small intestine and gut-associated lymphoid tissues, such as

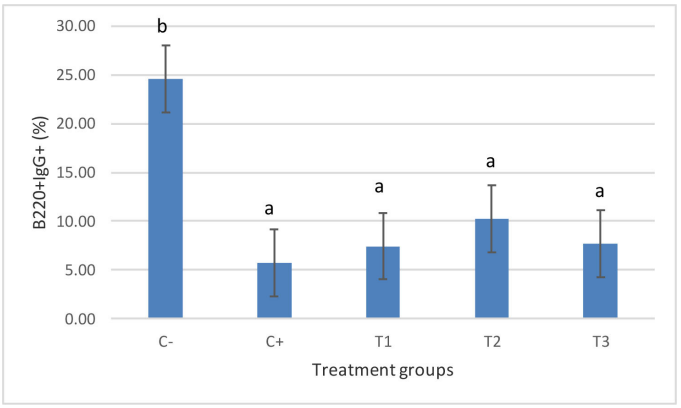


Figure 3. Treatment with LAB showed an increase in the relative number of CD4+CD8+ cells for all treatments, which did not differ significantly ($p < 0.05$). The treatment groups are: C- (healthy mice), C+ (mice induced with AFB1), and treatment groups, T1, T2, and T3 in which mice were administered with AFB1 and 1 × 105 CFU/ml, 1 × 107 CFU/ml, and 1 × 109 CFU/ml of LAB on days 7–28, respectively.

the Peyer's patches. Most dendritic cells (DCs) are present in an immature condition and are less immunogenic because of the low expression of MHC costimulators. Contact with pathogen-associated molecular patterns (PAMPs) or other signals induces the pattern recognition receptor (PRR) signal and activates the NF-κB pathway, resulting in maturation and activation of DC cells (Wells, 2011). Mature DCs may then express high MHC levels, costimulatory molecules, and cytokines which attenuate APC activation and differentiation of T cells to cause inflammation (Mohamadzadeh *et al*., 2005). DCs exposed to AFB1 cannot respond correctly to any invading microbes and fails to initiate antigen presentation to activated T cells that are susceptible to pathogenic agents (Mohammadi *et al*., 2014).

The anti-inflammatory cytokine, TGF-β, inhibits the proliferation of fibroblast epithelial cells, dendritic cells, and macrophages to produce inflammatory cytokines and controls cell growth through adhesion and extracellular matrix formation (Hussain *et al*., 2018). Exposure to mycotoxin could stimulate CD11c+ cells to activate inflammatory pathways that TGF-β may have suppressed. Oral exposure to LAB concentrations of 105 CFU/ml could activate inflammatory cytokines, such as TGF-β, in DCs, which may inhibit CD11c+ cell activity. This was evident in the T1 and T2 group, prior to an observed decrease in the T3 group. According to Vindirelo and Alberto (2015), the higher the concentration of cell bacteria, the greater the binding capacity of AFB1 in liquid media *in vitro*. The concentration of bacteria that can bind AFB1 was 1010 CFU/ml for *L. rhamnosus* GG, *L. casei Shirota*, *Propionibacterium freudenreichii* ssp. *shermanii* JS, and *Escherichia coli*. Probiotics have an immunomodulatory effect on the release of cytokines, interleukins, tumor necrosis factor, transforming growth factor, and chemokines from immune cells that play a role in the innate and adaptive immune systems. LAB may interact with enterocytes and DCs, Th1/Th2 cytokines, or T reg cells in the intestine to stimulate the adaptive immune response into a proinflammatory or anti-inflammatory action (Azad *et al*., 2018; Mohamadzadeh *et al*., 2005).

Mycotoxin AFB1 exerts toxicity because it is readily absorbed by the intestine and rapidly binds to serum protein. AFB1 is

genotoxic and immunogenic in animals (Zimmermann *et al*., 2014). Mycotoxin can activate the microbial intestine, and mycotoxin adsorption–desorption is highly dependent on the intestinal environment and digestive enzymes. The LAB, *L. rhamnosus* RC007, stimulates pH, salts, enzymes, and peristalsis at each stage of AFB1 absorption in the digestive tract. Saliva secretion results in low adsorption and high AFB1 reabsorption. Gastric fluids and intestinal fluids do not decrease the AFB1 adsorption of LAB, rather they stimulate higher AFB1 adsorption (Sadiq *et al*., 2019).

Metabolic LAB products inhibit aflatoxin biosynthesis. Heterofermented LAB, such as *L. bulgaricus*, produce a high level of acetic acid and propionic acid at acidic pH (Vinderola and Ritieni, 2015). The mechanism of action of LAB is to inactivate the fungal membrane and inhibit the absorption of amino acids and inactivated products from fungi, such as acetic acid (Perczak *et al*., 2018). Bacteria and yeasts may neutralize mycotoxins in the body by reshuffling, transforming, and breaking them down into nontoxic metabolic products or inactive forms (Murugesan *et al*., 2015). LAB binds mycotoxins to prevent further absorption by the intestine, which are then secreted with feces (Adilah *et al*., 2018). LAB walls contain peptidoglycans that could interact with mutagenic compounds, including mycotoxins, through binding to reduce stability and bioavailability, and stimulate the secretion of anti-inflammatory cytokines by macrophages (Niderkorn *et al*., 2009; Tabari *et al*., 2018). LAB's capacity to bind mycotoxins would be optimal when the bacteria cells die due to a change in the cell surface. Live LABs require a long time to release mycotoxin from the body (Perczak *et al*., 2018). Cell wall protein denaturation may function by creating a broader area to absorb mycotoxins (Tabari *et al*., 2018). The proteins in the ribosomes, nucleus, chromosomes, cytosol, and cellular cytoskeleton components support the forming of the cell wall of bacteria in the exponential growth phase.

On the contrary, binding between the cell wall of LAB with mycotoxin takes place at the beginning of the end of the bacterial growth cycle (Moller *et al*., 2021). AFB1 could bind to the cell wall β-d-glucan through hydrogen or van der Waals bonds. Absorption of AFB1 toxin depends on the availability of the number of binding sites on the surface of microbes, and the equilibrium constant [K (eq)], which could change as a result of genetic, physical, or chemical alterations (Sadiq *et al*., 2019).

LAB may act as an anti-inflammatory agent, resulting in the reduction of oxidative stress from AFB1 exposure (Abbes *et al*., 2016). Probiotics could also stimulate T cell subsets, humoral immune cells, epithelial-associated dendritic cells, and macrophages to increase anti-inflammatory cytokine products Braat *et al*., 2004. The entry of LABs into the body may increase the capacity and phagocytic receptors of leukocyte cells, especially complement receptor 3 (CR3), for bluffing respiratory bursts (Bravo *et al*., 2019).

Effect of LAB on the relative number of $CD4^+/CD8^+$ cells

Our results showed that the administration of LAB (*L. bulgaricus*) had an effect on the relative number of CD4+/CD8+ immunocompetent cells in mice induced with aflatoxin B1 in the treatment groups ($p > 0.05$) based on a Kruskal–Wallis test. The negative control group had a higher relative number of CD4+/CD8+ T cells than the positive group. This result is consistent with that of Qian *et al*. (2012), in which the administration of AFB1 to mice orally for 24 hours reduced CD3 T cells in the intestinal mucosa. AFB1 caused a decrease in the cellular immune response to the specific dose and duration treatments (Zimmermann *et al*., 2014). A low dose of mycotoxin could induce an inflammatory response if activated by enzymes, such as inflammatory inhibitors (Hussain *et al*., 2018). Mycotoxin AFB1 may undergo systemic hydrolysis and further activate metabolism. Phase metabolism includes conjugation with glucuronic acid and sulfate by the whole-cell biotransformation system during immune cell communication (Tran *et al*., 2020).

High LAB concentrations could increase naive T lymphocyte activation and proliferation as well as memory T cells (Wells *et al*., 2011). Upon entering the body, an antigen is presented by DCs in the digestive tract mucosa (CD11c). Histocompatibility complex class II molecules combined with the expression of costimulatory molecules and cytokines (Gaudinoand Kumar, 2019) and activation of T lymphocytes. As a cellular defense, T cells may differentiate into a subset of T1 that activates macrophages. Macrophages and APCs induce T cells to secrete inflammatory cytokines that cause tissue damage (Mohamadzadeh *et al*., 2005). The T1, T2, and T3 groups had a higher relative amount of CD4+/CD8+ T cells compared with the positive control group is shown in Figure 2.

LAB may act as an antifungal agent because it contains metabolites, such as organic acids, carboxylic acids, phenolic acids, cyclic dipeptides, hydrogen peroxide, and compounds that inhibit sporulation, which may decrease mycotoxin production (Sadiq *et al*., 2019). LAB at concentrations of 105 CFU/ml could activate T lymphocyte cells in all groups compared with the positive control group. These results were consistent with that reported by Tsai *et al*. (2012) in which LAB activates the cellular adaptive immune response.

THE EFFECT OF LAB ON THE RELATIVE NUMBER OF $B220^+IGG^+$ CELLS

Our results showed that $B220^+$ cells expressing IgG in the negative control group was higher compared with the positive group, whereas it was not significantly different among all of the treatment groups. The average of B220 cells expressing IgG increased after LAB administration. Mycotoxin can reduce immunoglobulin production in chicken feed supplemented with AFB1 (Nazarizadeh and Pourreza, 2019). LAB stimulates the humoral immune response by increased circulating antibodies and levels of plaque-forming cells in the host when exposed to mycotoxin (Abbes *et al*., 2015). LAB can absorb and eliminate mycotoxin to prevent intestinal absorption and reduce liver damage, which is a target of mycotoxin. Upon entering the host orally, mycotoxin stimulates the secretion of immunoglobulin A (IgA) in the digestive mucosa and antibody IgG in the circulation. IgG represents a secondary response to protect the body against foreign antigens (Chen and Tsai, 2011).

In the normal intestinal epithelium, microflora bacteria act as a barrier antigen. However, when epithelial cells are degraded by infectious and noninfectious substances, alterations in intestinal permeability and inflammation of the intestinal mucosa occur. The inflammatory response that occurs in the intestine activates IgG antibodies and causes translocation of the microflora bacteria (Paludan *et al*., 2020).

LAB plays a role in the body to activate the immune response by inducing the formation of Secretory IgA (SIgA) and producing vitamins (Wold, 2001). Antibody SIgA is dominant in the

mucous membrane, which is the first defense immune system against a dangerous environment. SIgA antibodies play a role in neutralizing toxins, viruses, salivary exotoxins, and eliminating pathogenic microbes (Hayati *et al*., 2018). *Lactobacillus* bacteria and other probiotics are commensal microorganisms that interact with the mucosa or the immune cells. LAB stimulates specific functions of the mucosal immune system and produces secretory IgA. The presence of receptors, such as Toll-like receptors, nucleotide oligomerization domain-like receptors, and C-type lectin receptors may stimulate *Lactobacillus*. *Lactobacillus* associates with microbe-associated molecular patterns to activate APC and modulate their function through the expression of surface receptors, secretion of cytokines and chemokines, and other nonspecific immune effector cells (Mohamadzadeh *et al*., 2005). Our results indicate that there was an increase in the relative number of IgG+ cells resulting from LAB induction in mice exposed to mycotoxin. This is consistent with the results obtained by Tran *et al*. (2020) in which IgG levels in the serum of Balb/c mice increased when *Lactobacillus* was administered for 7 days (infected with *Salmonella typhimurium* bacteria). DCs play a role in the adaptive immune response. LAB stimulates DC cells to activate specific immune responses in the intestinal mucosa to maintain homeostasis, protect against pathogenic microbes, and maintain intestinal permeability (Mohamadzadeh *et al*., 2005). LAB increases the cellular and nonspecific humoral immune response in mice exposed to mycotoxin AFB1.

CONCLUSION

L. bulgaricus bacteria exhibited a potent effect as an immunostimulator resulting from exposure to mycotoxin AFB1.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the ethics committee (certified no. 012-KEP-UB-2020) of Institut BioSains.

ACKNOWLEDGMENTS

The authors thank the Faculty of Science, Universitas Brawijaya, for processing samples by flow cytometry, and the Institut BioSains, Universitas Brawijaya, for maintaining the animals.

COMPETING INTERESTS

The authors declare that there are no financial and nonfinancial conflicts of interest.

FUNDING

The Faculty of Veterinary Medicine, Universitas Brawijaya, provided funding through a DPP SPP grant.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

AUTHORS' CONTRIBUTIONS

DQS obtained the funding; designed the study, analysis and interpretation of data; and was a major contributor in writing the manuscript. DQS, SM, and IAA analyzed the flow cytometer data, treated the animals, and collected the data. All authors read and approved the final manuscript.

REFERENCES

Abbès S, Salah-Abbès JB, Jebali R, Younes RB, Oueslati R. Interaction of aflatoxin B1 and fumonisin B1 in mice causes immunotoxicity and oxidative stress: Possible protective role using lactic acid bacteria. J Immunotoxicol, 2015; 13(1):46–54.

Adilah ZN, Liew WPP, Redzwan SM, Amin I. Effect of high protein diet and probiotic *Lactobacillus casei* Shirota supplementation in aflatoxin B1-induced rats. Biomed Res Int, 2018; 2018:9568351.

Ardiana O, Rifa'i M. The effect of dexamethasone treatment to humoral immunity in BALB / C mice models. J Biotropika, 2015; 3(3): 112–6.

Azad MAK, Sarker M, Wan D. Immunomodulatory effects of probiotics on cytokine profiles. Biomed Res Int, 2018; 2018:8063647.

Braat H, Van Den Brande J, Van Tol E, Hommes D, Peppelenbosch M, Van Deventer S. *Lactobacillus rhamnosus* induces peripheral hyporesponsiveness in stimulated CD4+ T cells via modulation of dendritic cell function. Am J Clin Nutr, 2004; 80(6):1618–25.

Bravo M, Combes T, Martinez FO, Cerrato R, Rey J, Garcia-Jimenez W, Fernandez-Llario P, Risco D, Gutierrez-Merino J. Lactobacilli isolated from wild boar (Sus scrofa) Antagonize Mycobacterium bovis Bacille Calmette-Guerin (BCG) in a species-dependent manner. Front Microbiol, 2019; 10:1663.

Chen CHC, Tsai YCC. Effects of multistrain lactic acid bacteria with probiotic properties on enhancements of IgA, IgG levels and anti-*Salmonella typhimurium* invasion activity. 2011; (34).

Gaudino SJ, Kumar P. Cross-talk between antigen presenting cells and t cells impacts intestinal homeostasis, bacterial infections, and tumorigenesis. Front Immunol, 2019; 10:360.

Hamidi A, Mirnejad R, Yahaghi E, Behnod V, Mirhosseini A, Amani S, Sattari S, Darian EK. The aflatoxin B1 isolating potential of two lactic acid bacteria. Asian Pac J Trop Biomed, 2013; 3(9):732–6.

Hayati M, Herman H, Rezano A. The effect of probiotic Lactobacillus casei supplementation on the secretory immunoglobulin A level in the saliva of wistar rats. Bali Med J, 2018; 7(3):727–31.

Hussain AF, Sulaiman GM, Dheeb BI, Hashim AJ, Alrahman ESA, Seddiq SH, Khashman BM. Histopathological changes and expression of transforming growth factor beta (TGF-β3) in mice exposed to gliotoxin. J King Saud Univ – Sci, 2020; 32(1):716–25.

Ibrahim AAE. Vitamin A downregulating Bcl-2 and TGF-α expression during colon cancer in AFB1-induced female rats. J Nat Sci Res, 2013; 3(5):67–84.

Møller GOD, Freire L, Rosim RE, Margalho LP, Balthazar CE, Franco LT, Sant'Ana AD, Corassin CH, Rattray FP, de Oliveira CAF. Effect of lactic acid bacteria strains on the growth and aflatoxin production potential of Aspergillus parasiticus, and their ability to bind aflatoxin B1, ochratoxin A, and zearalenone in vitro. Front Microbiol, 2021; 12:655386.

Mohamadzadeh M, Olson S, Kalina W V., Ruthel G, Demmin GL, Warfield KL, Bavari S, Klaenhammer TR. *Lactobacilli* active human dendritic cells that skew T cells toward T helper 1 polarization. Proc Natl Acad Sci USA, 2005; 102(8):2880–5.

Mohammadi A, Mehrzad J, Mahmoudi M, Schneider M. Environmentally relevant level of aflatoxin B1 dysregulates human dendritic cells through signaling on key toll-like receptors. Int J Toxicol, 2014; 33(3):175–86.

Muñoz R, Arena M, Silva J, González S. Inhibition of mycotoxinproducing *Aspergillus nomius* VSC 23 By Lactic Acid Bacteria and *Saccharomyces cerevisiae*. Brazilian J Microbiol, 2010; 41:1019–26.

Murugesan GR, Ledoux DR, Naehrer K, Berthiller F, Applegate TJ, Grenier B, Phillips TD, Schatzmayr G. Prevalence and effects of mycotoxins on poultry health and performance, and recent development in mycotoxin counteracting strategies. Poult Sci, 2015; 94(6):1298–315.

Nazarizadeh, H and Pourreza J. Evaluation of three mycotoxin binders to prevent the adverse effects of aflatoxin B1 in growing broilers. J Appl Anim Res, 2019; 47(1):135–9.

Niderkorn V, Morgavi DP, Aboab B, Lemaire M, Boudra H. Cell wall component and mycotoxin moieties involved in the binding of fumonisin B1 and B2 by lactic acid bacteria. J Appl Microbiol, 2009; 106(3):977–85.

Oswald IP, Marin DE, Bouhet S, Pinton P, Taranu I, Accensi F. Immunotoxicological risk of mycotoxins for domestic animals. Food Addit Contam, 2005; 22(4):354–60.

Perczak A, Goliński P, Bryła M, Waśkiewicz A. The efficiency of lactic acid bacteria against pathogenic fungi and mycotoxins. Arh Hig Rada Toksikol, 2018; 69(1):32–45.

Perdigón G, Fuller R, Raya R. Lactic acid bacteria and their effect on the immune system. Curr Issues Intest Microbiol, 2001; 2(1):27–42.

Pierron A, Alassane-Kpembi I, Oswald IP. Impact of mycotoxin on immune response and consequences for pig health. Anim Nutr, 2016; 2(2):63–8.

Qian G, Tang L, Wang F, Guo X, Massey ME, Williams JH, Philips TD, Wang J. Physiologically based toxicokinetics of serum aflatoxin B1-lysine adduct in F344 rats. Toxicology, 2013; 303:147–51.

Rohmawati E, Rifa'i M. Ethanol extracts of propolis (Eep) against lymphocyte activation cells in healthy mice (*Mus Musculus*) Balb / C. J Biotropika, 2014; 2(4):203.

Sadiq FA, Yan B, Tian F, Zhao J, Zhang H, Chen W. Lactic acid bacteria as antifungal and anti-mycotoxigenic agents: a comprehensive review. Compr Rev Food Sci Food Saf, 2019; 18(5):1403–36.

Tabari G, Kermanshahi H, Golian A, Heravi R. In vitro binding potentials of bentonite, yeast cell wall and lactic acid bacteria for aflatoxin B1 and ochratoxin A. Iran J Toxicol, 2018; 12(2):7–13.

Tomková I, Ševčíková Z, Levkut M, Revajová V, Čonková E, Laciaková A, Lenhardt L. Effect of aflatoxin B1 on CD3 T cells and alkaline phosphatase in the intestine of mice. Mycopathologia, 2002; 154(1):15–9.

Tran VN, Viktorová J, Rum T. Mycotoxins: biotransformation and bioavailability assessment using Caco-2 cell monolayer. Toxins, 2020; 12(10):628.

Vinderola G, Ritieni A. Role of probiotics against mycotoxins and their deleterious effects. J Food Res, 2014; 4(1):10.

Wells JM. Immunomodulatory mechanisms of lactobacilli. Microb Cell Fact, 2011; 10(Suppl. 1):1–15.

Wold AE. Immune effects of probiotics. Scand J Nutr, 2001; 45(2):76–85.

Zain ME. Impact of mycotoxins on humans and animals. J Saudi Chem Soc, 2011; 15(2):129–44.

Zimmermann C, Machado A, Cadoná F, Jaques J, Schlemmer K, Lautert C, Da cruz I, Zanette RA, Leal DBR, Santurio J. In-vitro cytotoxicity of aflatoxin B1 to broiler lymphocytes of broiler chickens. Brazilian J Poult Sci, 2014; 16(3):307–12.

How to cite this article:
Qosimah D, Murwani S, Amri IA, Anisa AK, Beltran MAG. Regulation of the immune system by administering lactic acid bacteria to suppress the effects of aflatoxin B1 in mice (*Mus musculus*). J Appl Pharm Sci, 2022; 12(07):184–189.

World Journal of Engineering Research and Technology
WJERT
www.wjert.org
SJIF Impact Factor: 5.924

SITE SUITABILITY ASSESSMENT AND CLIMATE VULNERABILITY FOR SWEETPOTATO IN MONCADA, TARLAC, PHILIPPINES

RJ. P. Tungpalan[1], E. D. Galo[2*], G. B. Damian[2] and MG. N. Semilla[2]

[1]Rootcrops Research Training Center, Tarlac Agricultural University, Philippines.
[2]Department of Agricultural and Biosystems Engineering, Tarlac Agricultural University, Philippines.

Article Received on 30/04/2022 **Article Revised on 21/05/2022** **Article Accepted on 10/06/2022**

***Corresponding Author**
E. D. Galo
Department of Agricultural and Biosystems Engineering, Tarlac Agricultural University, Philippines.

ABSTRACT

Sweetpotato is a very resilient crop, needs plenty of sunshine, can tolerate drought to some extent but cannot survive water logging, on the other hand, storage roots are sensitive to changes in soil temperature, depending on the stage of its root development. Majority of the farm areas in sweetpotato production are lowland to hilly in which some of the farm lands are highly affected by flood, drought and erosion. Thus, there is a need to assess areas which are vulnerable to climate changes. Moreover, there is a need to evaluate possible suitable areas to meet the increasing demands in sweetpotato delicacies. To address this problem, identifying suitable sites for sweetpotato production and generating climate vulnerability maps could help farmers and local government units (LGU) to assess the proper use of different resource maps for decision making and planning. Five parameters (land use, soil type, groundwater, slope and road accessibility) were used in suitability assessment while the bioclimatic factors, hazards and the existing sweetpotato areas were used for the climate vulnerability analysis. Data were gathered from different agencies and field survey that was processed using MaxEnt and GIS software. Based on the result, majority of the municipality were suitable to highly suitable for sweetpotato production. On the other hand, climate vulnerability assessment shows that the effect climate and hazards to these areas was moderate to extremely vulnerable to these changes in the coming years.

KEYWORDS: Site assessment, Climate vulnerability, Sweetpotato.

1 INTRODUCTION

Sweetpotato is an important staple and emergency food in many countries and is appreciated for its very high nutritional value, both of the tubers and of the young aerial parts.[1] It is also considered as a vegetable, a snack food, ingredients in animal diets and now being used for processed products. Sweetpotatoes are of tropical origin, warm climates is well adapt and grow best during summer. A well- drained sandy loam is desired and heavy clay soils should be avoided as they can delay root development, causing in growth cracks and poor root shape. Sweetpotato needs plenty of sunshine, but shade causes yield reduction.[2] Sweetpotato can tolerate drought to some extent but cannot survive water logging.[3]

The Philippines is reported as one of the most affected countries in terms of climate related risks to agriculture.[4] Crops are both affected by extreme weather as well as the different climate hazards. These include typhoon, landslides, flooding and soil erosion and the magnitude and incidence of these hazards is projected to rise under a climate change scenario.[5] Rainfall is becoming more variable and temperatures are rising consequently leading to increase occurrence of droughts and floods, and changes in the timing and length of growing seasons.[6] Exposure and sensitivity together describe the potential impact that climate change can have on a system.

Site specific sustainable agro-techniques through well designed research is important to improve sweetpotato productivity. Areas, where sweetpotato is already staple food has great potential to improve the farming practices. Thus, identifying a suitable site for sweetpotato production and generating a climate vulnerability map could help farmers and Local Government Units for decision making and planning.

2 MATERIALS AND METHODS

2.1 Study Area

The area for this study focused on the land of Tarlac Province where the total area of 3,053.45 km^2 (305,345 ha) with 37 barangays (Figure 1). Tarlac has dry and wet season. Sweetpotato is the pride of Tarlac, as the province is one of the largest commercial producers of rootcrop in the country. In this study, the three (3) barangay with the largest areas in the municipality was chosen.

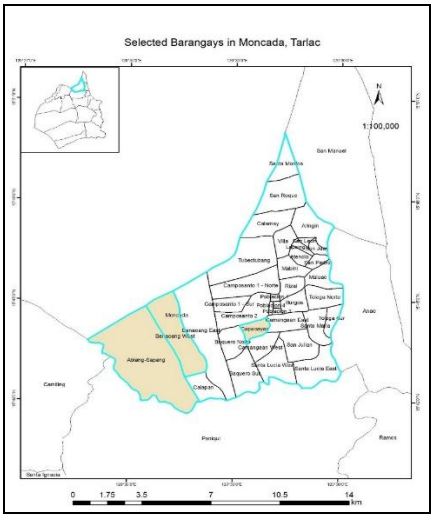


Fig. 1: Map of Moncada, Tarlac, Philippines.

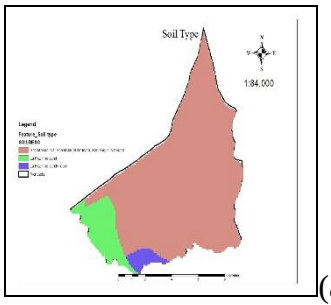
2.2 Site Suitability Analysis

Secondary data and demographic data was collected from different Government organizations. Primary data was gathered through key informant interviews (KIIs) and focus group discussion (FGDs) in relation to sweetpotato areas and the existing farmer practices and management in sweetpotato production.

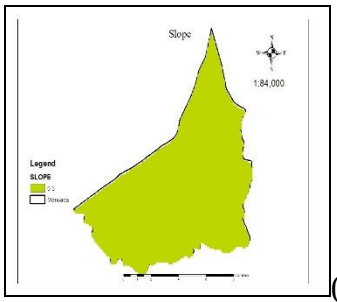
The five parameters used for the site suitability analysis were processed and analyzed using ArcGIS software. The given parameters are the soil type, slope, land use, groundwater and road accessibility (Figure 2).[7] The identified categories of each parameters have a different score based on its suitability.

The site suitability scoring and weighting system was based in five different parameters with suitability scoring (Table 1). Developing the suitability map of the municipality were done by overlaying the reclassified suitability parameters with their corresponding weights using the Equation 1. Then, it was classified into five suitability classes (Table 2).

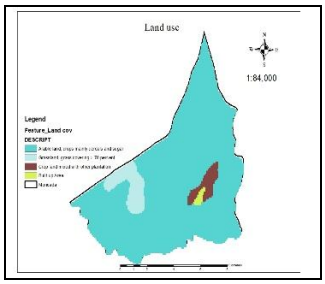
Suitability Score = (Land use)*30 + (Soil type)*10 + Groundwater)*5 (1)
+ (Slope)*3 + (Road accessibility)*2



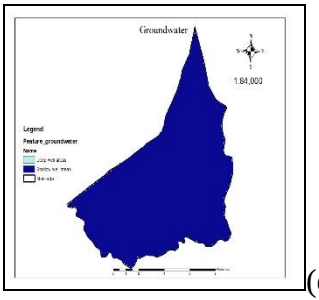
(a)



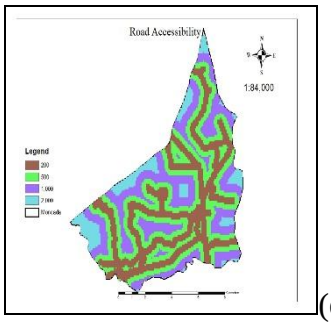
(b)



(c)



(d)



(e)

Fig. 2: Site Suitability Parameters Maps (a) Soil type Map (b) Slope Map (c) Land use Map (d) Groundwater Map (e) Road accessibility Map.

Table 1: The Suitability Scoring and Weighting.

Parameters	Category	Scoring	Weighting
Land use	Arable land Grassland, grass covering >70% Build-up area	10 7 0	30
Soil type	Sandy loam Clayloam Fine sand Other types	10 8 5 1	10
Groundwater	Deep well areas Shallow well areas	10 7	5
Slope	0-3 3-8 8-15 15-30 30 and up	10 8 6 2 0	3
Road accessibility (Buffer, meter)	0-200 200-500 500-1000	10 7 4	2

Table 2: The Suitability Classes.

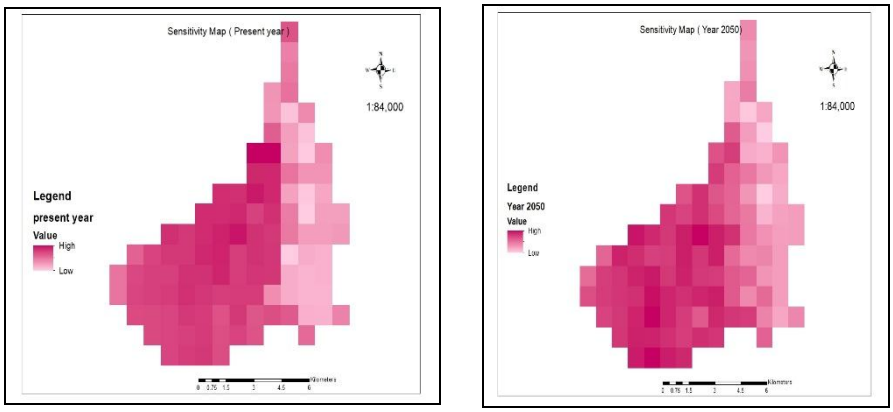
Class	Suitability Score
Highly Suitable	14-13.0001
Moderately Suitable	13-12.0001
Suitable	12-11.0001
Less Suitable	11-10.0001
Not Suitable	10-0.0000

2.3 Climate Vulnerability (Sensitivity-Hazard) Analysis

Sensitivity and exposure together describe the potential impact that climate change can have on a system. This was done by overlaying the sensitivity and hazard within the municipality.

Exposure I. Sensitivity analysis (changes of temperature and precipitation)

Sensitivity analysis was done by identifying the existing crop distribution through focus group discussion in participation of the Municipal Agriculture staff and sweetpotato farmers and the incorporation of bioclimatic data (temperature and rainfall) from the WorldClim. This was processed on the MaxEnt software, a species distribution model (SDM)[4] to produce the sensitivity map (Figure 3).



(a) **(b)**

Fig. 3: Sensitivity Map (a) Sensitivity Map of Present Year, (b) Sensitivity Map of Year 2050.

Exposure II. Hazards

Exposure represents the climate conditions that stimuli against extreme changes. The hazards such as typhoon, flood, erosion and drought (Figure 4) was considered which mostly affects the area. Secondary data on these hazards were gathered from different organizations and agencies. Hazard weights identification (Table 3) were participated by PDRRMC/MDRRMC staff and AEWs.

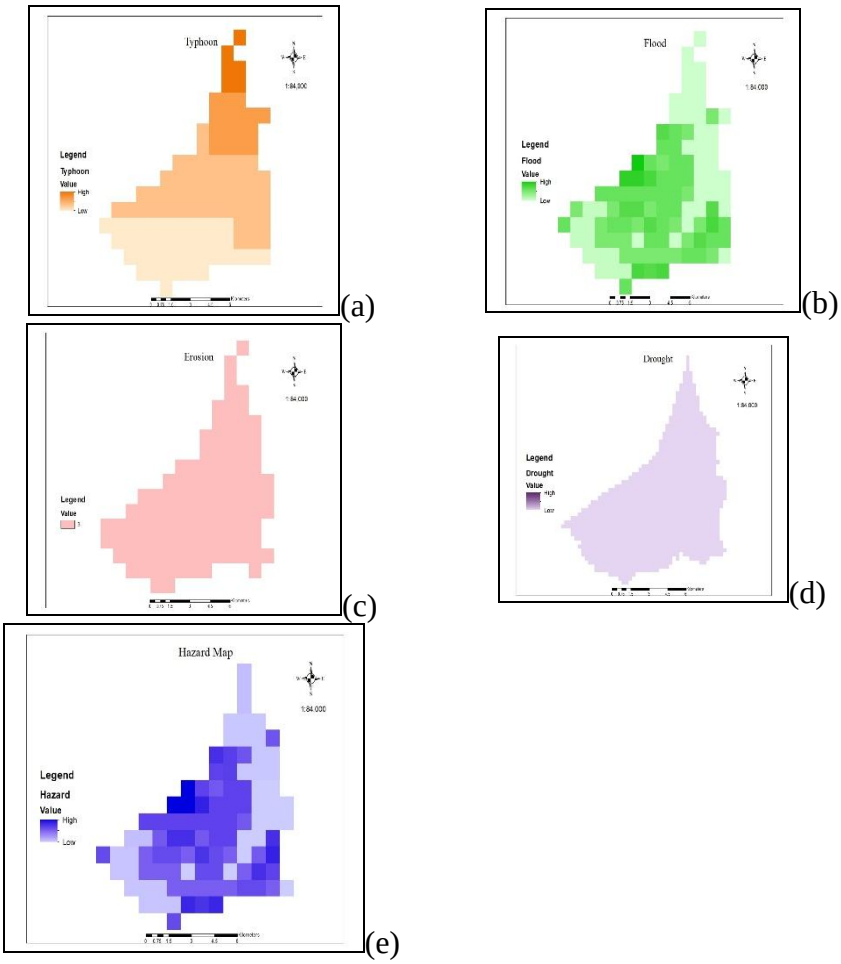


Fig. 4: Hazard Maps (a) Typhoon Map (b) Flood-prone Map (c) Erosion Map (d) Drought Map (e) Final Hazard Map.

Table 3: Hazard Weights.

Exposure II. Biophysical	Probability of Occurrence	National Economy	Food Security	Houshold Income	Key Natural Resources to Sustain Productivity	Weight
Typhoon	3	3	4	4	1	15
Flood	3	4	4	4	1	16
Erosion	1	1	1	1	1	5
Drought (Agricultural/Hydrological)	3	3	3	3	3	15

Note: Weighting the natural hazards into a climate risk exposure

Probability of occurrence: 1 in 1 year = 5, every 5 years = 3, 1 every 10 years = 1

Impact: Insignificant = 1, minor = 2, moderate = 3, significant = 4, disastrous = 5

3 RESULTS AND DISCUSSION

3.1 Sweetpotato Production Areas

The largest area for sweetpotato production in province of Tarlac is Moncada. Three barangays in Moncada were selected in the inventory of the existing crops. The largest area with sweeetpotato production is Ablang Sapang followed by Banaoang West and lastly, Capaoayan having a total farm area of 352.8 hectares with 317 number of famers, 50.9 hectares with 57 numbers of farmers and 3.5 hectares with 2 numbers of farmers, respectively (Figure 5).

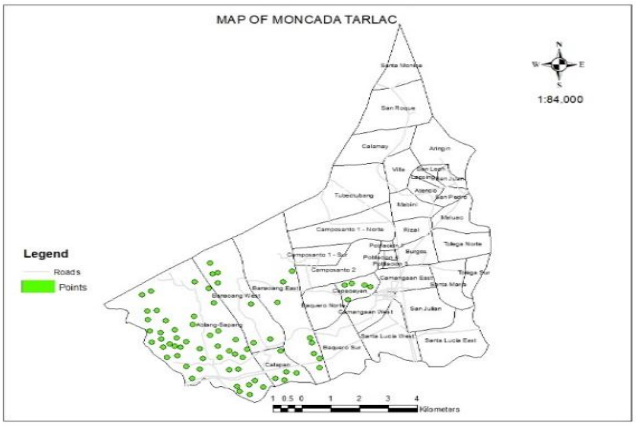


Fig. 5: Sweetpotato Production Areas with 3 Selected Barangays.

3.2 Site Suitability Map

The suitability map shows that the area in Municipality are classified as moderately suitable to highly suitable for sweetpotato production (Figure 6).

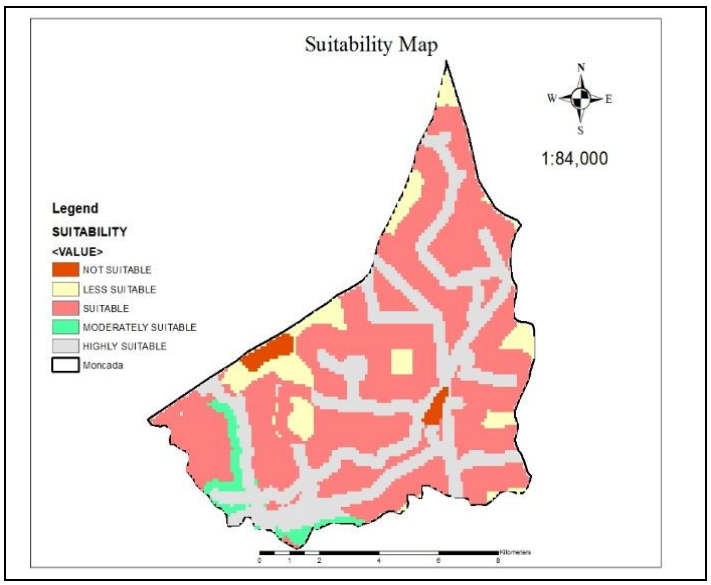
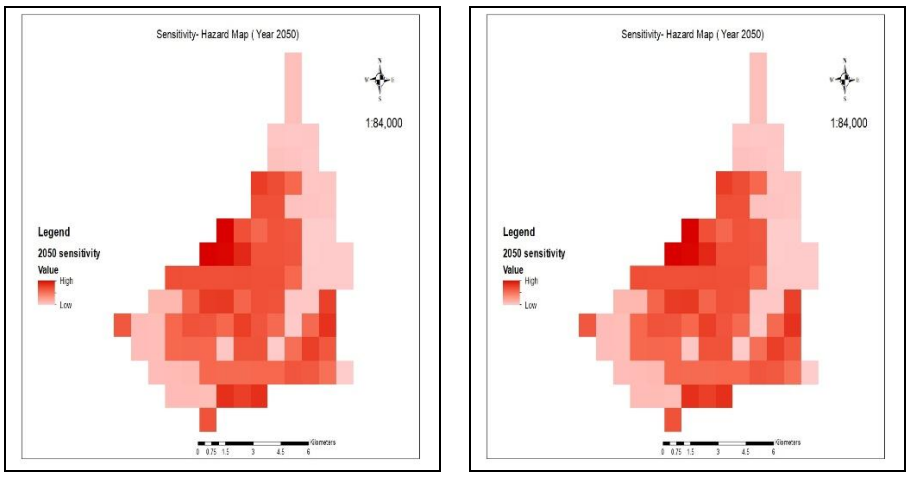


Fig. 6: Site Suitability Map of Sweetpotato in Moncada, Tarlac, Philippines.

3.3 Climate Vulnerability (Sensitivity-Hazard) Map

The impact of the sensitivity-hazard from the present up to year 2050 is moderate to extreme because of the changes in climate (Figure 7). This means that area is vulnerable for crop production.



(a) **(b)**

Fig. 7: Sensitivity-Hazard Map of Sweetpotato in Moncada, Tarlac, Philippines.

4 CONCLUSION

The area of the selected barangays in this study were found out that it is highly suitable, moderately suitable and suitable for sweetpotato production based on the parameters. The information of the impact of climate in the area is also identified together with the hazards defining the risks posed by climate change. This study provides information on sweetpotato that can be used for identifying measures to adapt to climate change impacts.

REFERENCES

1. Maria, D. and Rodica, S.: Researches on the sweetpotato (*Ipomea bataas L.)* behavior under the soil and climatic conditions of the South west of Romania. Journal of Horticulture, Forestry and Biotechnology, 2015; 19(1): 79-84.
2. Nedunchezhiyan M, and Ray RC. Sweet potato growth, development production and utilization: overview. In: Ray RC, Tomlins KI (Eds) SweetPotato: Post Harvest Aspects in Food, Nova Science Publishers Inc., New York, 2010; 1-26.
3. Nedunchezhiyan M, Rajasekhara Rao K, Laxminarayana K, and Satapathy BS. Effect of strip intercropping involving sweet potato (*Ipomoea batatas L.)* on soil moisture conservation, weevil infestation and crop productivity. Journal of Root Crops, 2010; 36(1): 53-58.

4. Paquit, J.C., Bruno, AG.T., Rivera, TA.S., and Salingay, R.O.: Climate-risk vulnerability assessment of the agriculture sector in the municipalities and cities of Bukidnon, Philippines. International Journal of Biosciences, 2018; 13(6): 155- 168.
5. Field, CB. Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation: Special Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, 2012.
6. Ddumba, S. D. Assessing the impact of climate change and variability on sweetpotatoes in Eat Africa. 94th American Meteorological Society Annual Meeting. ams.confex.com, 2014.
7. Jain, K and Subbaiah, Y.V. Site Suitability Analysis for Urban Development using GIS. Journal of Applied Sciences, 2007; 7(18): 2576-2583, 2007.ISSN 1812-5654.

Nitrogen Deficiency Mobile Application for Rice Plant through Image Processing Techniques

Geraldin B. Dela Cruz

Abstract: ***Driven by the opportunity that digital devices and robust information are readily available, the development and application of new techniques and tools in agriculture are challenging and rewarding processes. This includes techniques learned that is based on traditional methods, practices, experiences, environmental patterns and human capability. The most sought technique comes from human intelligence that is dynamic, adaptive and robust. Nitrogen deficiency in rice plants can be determined via the color of the leaves. It is dependent on the depth of the green pigment in the color spectrum present in the leaves. Based on these characteristics, the application of computational artificial intelligence and machine vision can be adopted to create assistive technologies for agriculture. In this paper, a mobile application is developed and implemented that can be used to assist rice farmers determine nitrogen deficiency, through the leaf color in rice plants. The application can be used alternatively or together with the traditional protocol of nitrogen fertilizer management. It is mobile, simple and it also addresses some drawbacks of the human eye to distinguish color depths brought about by other factors, like sunlight, shading, humidity, temperature, etc. It utilizes image processing techniques to digitally captured images represented in numerically transformed Red, Green, and Blue color formats. The digital images are then normalized to remove the effects of illumination and then compared using the image/pixel subtraction technique with the base color images converted and extracted from the leaf color chart standard. Eventually, the application determines nitrogen deficiency and suggests the concentration and volume of fertilizer to be applied to the rice plants. Accuracy of the technique is determined by computing the Z statistic score.***

Keywords**: *Algorithms, image processing, fertilizer management, mobile application.***

I. INTRODUCTION

Fertilizer management is governed by processes triggered by specific events and attributes from the environment and most especially from the crop. The method is based on a standard protocol developed by researchers together with the farmers with years of tests and trials. This fertilization protocol is a tedious activity especially for the rice (*Oryza Sativa L.*) plant, it is not as easy as just throwing nutrients into the soil and everything will just be fine. There are some issues to be considered, such as applying too much fertilizer and the plant becomes succulent and susceptible to insect and disease. Too little and the plant grows poorly and unproductive. In the Philippines, majority of the farmers cultivate their farms the traditional way. These farmers apply fertilizers not only based on plant condition but also take into consideration predetermined dates after seeding or transplanting. Not following holistically the protocols established for fertilizer management, farmers suffer the consequences of bad fertilizer management, thus lesser harvest yield. Fertilizers must be applied only when necessary and based on the crops' nutritional status. However, most farmers rely on the age (days after transplanting) of the rice plant and not on its condition. Consequently, this causes a deficiency in the required nutrient of a plant from the fertilizer in terms of growth, development, and yield. Moreover, there are some unaware farmers, that applying fertilizer too soon, will result to undesirable effects on growth and yield of rice and thus have a significant addition to the production cost which is not ideal [1].

II. RELATED WORKS

There have been many developed methods of the proper application of fertilizer [2]. One of the most effective means to determine the volume and when to apply fertilizer is to use the developed Leaf Color Chart (LCC). The LCC is used to assess the plant Nitrogen (N) status. It is an inexpensive tool consisting of four (4) color shades from yellowish green to dark green. The color strips are fabricated with veins resembling those of rice leaves. The assessment will depend on the greenness of the leaf matched to the LCC window. Each window defines a level of N status. This method however, limits the capability of the human eye to distinguish from the colors given in the chart from the colors of the rice plant leaf as evidenced in the findings of the on-farm evaluation. The color matching is relative to the person's color perception so it is recommended that the same person should do the matching. The use of the LCC is also limited to a period of a day due to the effect of sunlight to the colors, both of the leaf and the chart [3], [4].

In the Philippines, the on-farm evaluation of the LCC technique has demonstrated its usefulness for real-time nitrogen management in rice. The increase in N-use efficiency was due to slightly less, same or higher yields grain, with lower levels of N application in the LCC-monitored fields. Savings in N fertilizer of -14 to +53 kg per hectare were realized in farmers' fields of other collaborating countries [5].The work of P. Sanyal and U. Bhattacharya explained that rice deficiencies in the balance of mineral levels can be identified by detecting the change in the appearance of rice leaves [6]. This work is also supported by P. Murakami et al, that changes in foliar color are a valuable indicator of plant nutrition and health.

Revised Manuscript Received on August 20, 2019.
***** Correspondence Author
Geraldin B. Dela Cruz*, College of Engineering and Technology, Tarlac Agricultural University, Camiling, Tarlac, Philippines. Email: delacruz.geri@gmail.com

The leaf color is measured with visual scales and inexpensive plant color guides that are easy to use, but not quantitatively rigorous, or by employing sophisticated instrumentation including chlorophyll meters, reflectometers, and spectrophotometers that are costly and may require special training [7].

The International Rice Research Institute (IRRI), and the Department of Agriculture (DA) in the Philippines, initiated the NM Rice Mobile application. It applies the concept of Site Specific Nutrient Management (SSNM), a set of scientific principles for optimally supplying rice with essential nutrients. The LCC is covered by SSNM. Farmers dial a toll-free number and a voice response will follow which will direct them to a set of 12 to 15 questions related to the status of the rice plant. Eventually, a text message will be sent to the farmer's phone containing recommendations on fertilizer application duly customized for his field. The mobile application is available in Tagalog, Cebuano, and Ilocano dialects [8].

The paper of S. Pongnumkol, P. Chaovalit and N. Surasvadi, presented a review of the capability of smart phones to becoming a very useful tool in agriculture, mainly to their mobility that matches the nature of farming, the cost efficiency and accessibility of computing power. It systematically reviewed smart phone applications that utilize built-in sensors to agricultural solutions [9].

Similarly, the work of V. Patodkar et al, presents a developed android software application for sustainable development for farmers. The application assists the farmer in decision making regarding selection of fertilizer, pesticide and time to do particular farming actions. It combines internet and mobile communications with Global Positioning System (GPS) [10].

The system developed by Sanjana, Sivasamy, Jayanth [11] consisted of a mobile application which enables farmers to take digital images of plants using their mobile phones and send it to a central server where the central system analyzes the pictures based on visual symptoms using image processing algorithms to measure the disease type. An expert group will be available to check the status of the image analysis data and provide suggestions based on the report and their knowledge, which is then sent to the farmer as a notification in the application.

Based on the insights from these pieces of literatures, this project aims to apply digital processing techniques in a mobile application that can be used as a tool to assist rice farmers in fertilizer management of the rice plant based on the LCC framework and its guidelines.

The project intends to implement the image normalization technique as a preprocessing method and the digital image pixel subtraction technique as the processing algorithm into a mobile phone application [12]-[14].

The application is to be used in the rice paddy field as an assistive technology for rice plant farmers. It aims also to archive data sampled from the rice field to be used as baseline comparative statistics by other researchers. The framework of the study was inclined on the use of smart mobile phone technology, image processing and rice farming technologies.

III. SYSTEM ARCHITECTURE

A. Application architecture

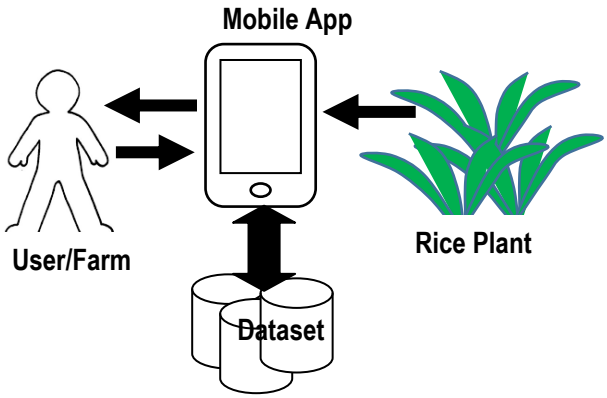


Fig. 1.A system view of the application.

Shown in Fig. 1, is the conceptual framework of the system: the mobile phone application processes sample images of leaves taken from rice plants in the field through its built-in camera device, these images are processed and eventually references the average image against the digitized LCC dataset. This process determines the nitrogen deficiency of the rice plant. The number of samples is dependent on the size of the rice field. Ideally, more samples from a large area, the better the outcome of the mobile application. However, the LCC standard suggests that five (5) leaf samples per hectare taken randomly from the field are sufficient to represent the whole area.

The intelligence of the application relies on the digitized LCC dataset that is used as the basis in determining the nitrogen content of the captured rice plant leaf color. The whole process is integrated into the mobile application: the user launches the application and through the camera of the phone, to take samples of the rice plant leaf. The application converts the images one at a time, calculates the average of the samples and performs image comparison. The method uses the color depths of the captured image from the base image and compares it from the digitized LCC database. Subsequently, the application displays the result, suggesting the amount of fertilizer to be applied. The results are archived in the database for future reference and further study.

B. Computational processing of the application

Fig. 2 presents the computational algorithm of the mobile application. The system takes sample images of rice leaves in the rice field. These images are then processed by converting it to its equivalent Red, Green, Blue (RGB) formats. By subtracting the average value of the sample images from the value of the baseline LCC images present in the application, consequently, a resulting near accurate color value is returned. If the sample images are out the range, then the user is alerted that the image is not a rice leaf. The same procedure is done until the captured image is valid. While the application captures the images, it also records the ten greenness values of the rice leaves.

The average greenness values of the ten images are also stored. The average greenness value of the sample images is then subtracted from the baseline values per window of the digitized LCC. After this process, the indicative result based on the interpretation of the greenness value is displayed. Included in the indicative result is the fertilizer recommendation accordingly to the specified window.

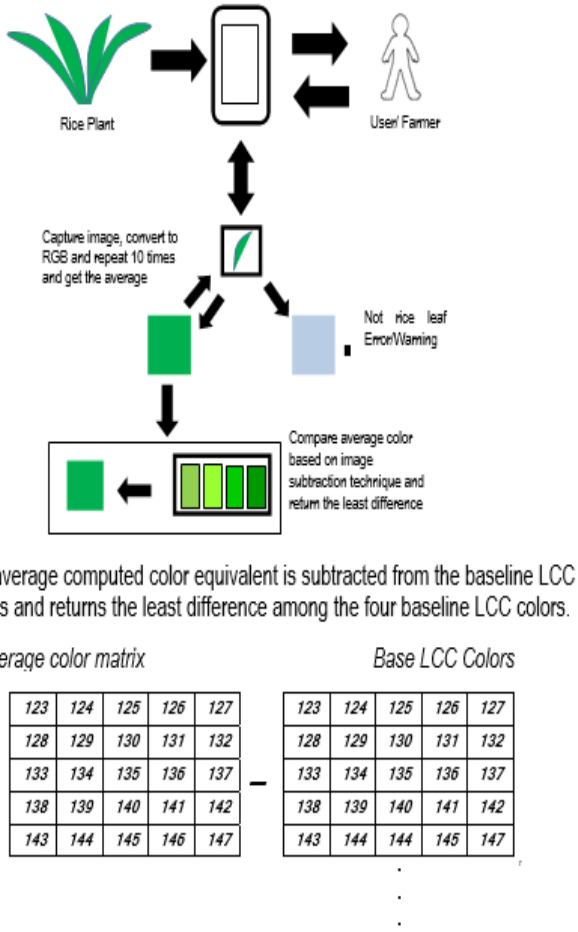


Fig. 2. Processing mechanism of the mobile application.

The RGB color space of the captured bitmap image is used as the numerical representation of the image. The RGB data value of each pixel's color sample has three numerical values to represent the colors Red, Green, and Blue. These three RGB components are three 8-bit numbers for each pixel. Each 8-bit RGB component can have 256 possible values, ranging from 0 to 255.

To get the area of concern from the image, the height and width of the bitmap is first calculated, which is denoted by:

$$Z = (0...x, 0...y) \quad (1)$$

Where:
Z = bitmap image
x = x coordinate plane
y = y coordinate plane

The color value of each pixel is represented in (2) denoted by:

$$P_{(x,\,y)} = (R, G, B) \quad (2)$$

Where:

$P_{(x,\,y)}$ = pixel in the x and y coordinate plane
(R,G,B) = (0...255, 0...255, 0...255)

Color normalization is also applied to the pixels to reduce the effects of light. Normalization of the color space of the image removes highlighted regions and shadows this makes it easier to detect the color of the leaf. Based on equation (2), the normalization method is presented below:

$$Total = (R + G + B) \quad (3)$$

$$R' = round((R / Total) * 255) \quad (4)$$

$$G' = round((G / Total) * 255) \quad (5)$$

$$B' = round((B / Total) * 255) \quad (6)$$

Thus, the normalized images are denoted by the equation in (7):

$$P1 \mid 2_{(x.y)} = (R', G', B') \quad (7)$$

The pixel subtraction technique is as simple as taking two images as input parameters, this mechanism produces a third image whose pixel values are simply the difference of the corresponding pixel values from the two images. It is also often possible to just use a single image as input and subtract a constant value from all the pixels. Some versions of this technique produce the absolute difference between pixel values, rather than the straightforward signed output.

The subtraction of two images can be performed straightforwardly in a single pass using the formula in equation (8).

$$[Q_{(i,\,j)} = P1_{(i,\,j)} - P2_{(i,\,j)}] \quad (8)$$

Where :
Q = the output value
P1 = the first image value
P2 = second image value

Or the absolute differences between the two input images can be computed from equation (9).

$$[Q_{(i,\,j)} = \mid P1_{(i,\,j)} - P2_{(i,\,j)} \mid] \quad (9)$$

Or simply subtract a constant value C from a single image if desired using the formula in equation (10):

$$[Q_{(i,\,j)} = P1_{(i,\,j)} - C] \quad (10)$$

Where:
P1 = first image value
C = baseline image value

The green (G) color component value in a pixel is simply extracted separately to produce the nearest output value.

IV. RESULTS

A. Detection Testing

Tests were conducted on the premise that the algorithm may result and interpret colors from other leaves other than of the rice leaf. Thus, a mechanism was integrated to accurately identify whether the captured image is that of a rice leaf.
Shown in Fig. 3, is the result after capturing thru the mobile phone camera the rice leaf. The image presents the confirmation of the object being a rice leaf which does not display warning or notification of an error. The application converts the image and saves it in RGB format

Fig. 3. Correct rice leaf image.

Fig. 4 and Fig. 5 shows the status screen of the mobile application when a different leaf or an object with the same color of a rice leaf is captured. It is capable of determining that the image taken is not a rice leaf, resulting in the notification display of an error-warning to the user. Not only the difference in color but also the difference between the two objects can be detected, even though the object captured has a similar color with a rice leaf. Fig. 6 shows the indicative results when the application correctly determines the captured image that is of a rice leaf. Consequently, the result of the detection process is displayed. In this case, the leaf is in category 4 of the LCC, which means the plant requires a certain amount of fertilizer. The application will also display the required volume of the fertilizer that should be applied in the rice field.

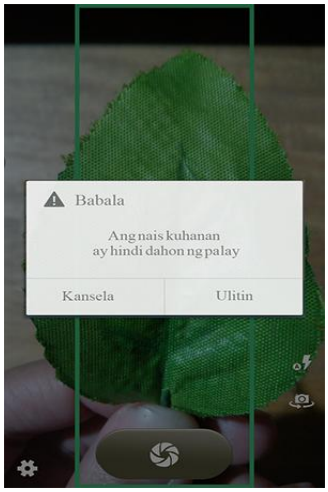


Fig. 4.Error detection warning for a different leaf with similar color.

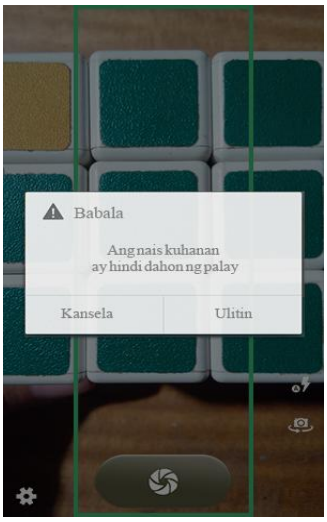


Fig. 5.Error detection warning for a different object with a green color.

Fig. 6.The indicative result when fertilizer deficiency is detected from the rice leaf.

Fig. 7.The indicative result when rice the plant is not fertilizer deficient.

Fig. 7 shows the result when the processed data from the captured image is within category 5 of the LCC, which means, not fertilizer deficient. The application will display a notification to the user that there is no need for fertilizer to be applied to the rice field.

B. Actual Field Test Results

The test data were gathered from the five (5) week actual field testing. The process was synchronized with the growth stages of the rice plant, to get the real colors of the rice plant throughout its different growth stages. The Z-test statistic was used to verify and validate the hypothesis that the developed mobile application using the image subtraction technique does not have a significant difference with the traditional LCC.

The field test consisted of a rice field of approximately three hectares. The area was divided equally into three zones (Area 1, Area 2 and Area 3) due to the geographical contours and for an equal number of samples per area sampled. Thirty (30) leaf sample pictures were taken from each area randomly, these thirty samples were also divided into three (3), so that ten (10) leaf samples for each strip one area, for a total of ninety (90) leaf samples each week.

The field test was done from the vegetation and milking stages of the rice plant, giving a total of 450 leaf samples, with an average of 45 samples. During the field tests, the researchers also synchronized the use of the traditional LCC. This was done so that readings are consistent with the LCC due to the leaf's condition for a short time. This is to lessen the effect of other factors like sunlight, moisture, wind, temperature, shading, etc.

Table- 1: The success rates of the system in the field test

Week	Area 1			Area 2			Area 3		
	a	*b*	*c*	*a*	*b*	*C*	*a*	*b*	*c*
1	6	6	6	8	8	8	6	6	6
2	6	6	6	8	8	8	6	6	6
3	6	6	6	8	8	8	6	6	6
4	7	7	7	7	7	8	9	7	9
5	7	7	7	8	8	8	9	7	10

[a] *$n = 45$ $\bar{x} = 7.08$ $\sigma = 0.03$

Table 1, shows the success rates readings of the mobile application in comparison to the LCC. The study assumed that the null hypothesis is equal to, Ho = 5, which is the mean success rate of the mobile application and the alternate hypothesis is greater than H1 > 5. To test the hypotheses if the application has no significant difference between the traditional LCC, the z-test statistics is employed.

To compute for the z-test statistic the formula in equation (11) is used. The alpha level considered by defaults is 5% (0.05). The rejection region area in the z-table is 0.05, which is equal to a z-score of 1.645.

$$Z = \frac{\bar{X} - \mu_0}{\sigma / \sqrt{n}} \tag{11}$$

Where:

Z = the test statistic, $\bar{x}$ = mean score, σ = standard deviation, n = population, sample, and μ_0 = null hypothesis

Continuing with the computation, the equation is used straightforward. The Z statistic value is then derived in (12):

$$Z = \frac{7.08 - 5.0}{0.03 / \sqrt{45}} = 1.033 \tag{12}$$

Comparing the computed Z-statistic test result score of 1.033 with the z score of 1.645, it shows that the computed Z statistic test score is less than the Z score prescribed in the Z table. This suggests that the null hypothesis is not rejected. Further, the results imply that using the mobile application can be an effective assistive technology for rice farmers and as efficient as the LCC. The accurateness of the system is assured as it has been proven thru statistical analysis that the mobile application does provide significant and similar results compare to the traditional LCC.

V. CONCLUSION

A mobile application was developed and the proposed method was successfully implemented. The results of the field experiment demonstrated that machine vision can be a tool to assist farmers in detecting the level of nitrogen deficiency of rice plant, by implementing image processing techniques as the mechanism. Specifically, the intelligence of the developed system is the application of the image or pixel subtraction algorithm. By using digitally captured bitmap images with their corresponding RGB numerical formats. This technique was proven to be easily executed as a function in the application, using an android based smart phone.

Field test results suggested that the developed mobile application is comparable to the traditional LCC standard. Meaning, they are complementary with each other or can be used individually without a significant difference in their outputs. Similarly, the statistical test result also implies that machine vision can be used as an assistive technology to rice farmers, specific to the detection of nitrogen deficiency of rice plants presented in this study. The implemented detection algorithm for nitrogen deficiency is accurate and efficient. Future endeavors to include other variables like temperature, time of the day, and age of the plant may be considered for the improvement of the application. To cover a larger area and for faster acquisition of images, an unmanned aerial vehicle is also being considered.

REFERENCES

1. A. Strub, Q. Kettirings, M. Hunter, K. Czymmek and T. Kilcer, "Basics of Fertilizer Management", in Agronomy Fact Sheet Series, 2012, Fact Sheet 75.
2. J.-S. Lee, Y.-S. Song, Y.-J. Lee, H.-B. Yun, B.-C. Jang, and R.-Y. Kim, "Effects of Customized Fertilizer Application on Growth and Yield of Rice," *Korean Journal of Soil Science and Fertilizer*, vol. 44, no. 6. pp. 1124–1129, 2011.
3. N. Ahmad, A. Zada, M. Junaid, and A. Ali, "Bridging the Yield Gap in Rice Production by Using Leaf Color Chart for Nitrogen Management," *Journal of Botany*, vol. 2016. pp. 1–6, 2016.

4. S. Vaseghi, M. Valinejad, and M. Afyuni, “Improvement of Nitrogen use Efficiencies using Leaf Color Chart (LCC) in Rice Field,” *Journal of Water and Soil Science*, vol. 21, no. 3. pp. 193–204, 2017.
5. V. Balasubramanian, A. C. Morales, R. T. Cruz, N. N. De, P. S. Tan, Hla Tin, and Z. Zaini. "Leaf color chart (LCC): a simple and inexpensive decision tool for the real-time nitrogen management in rice." *Philippine Journal of Crop Science Philippines*, 2000.
6. P. Sanyal, U. Bhattacharya, S. K. Parui, S. K. Bandyopadhyay, and s. Patel, “Color texture analysis of rice leaves diagnosing deficiency in the balance of mineral levels towards improvement of crop productivity”, in IEEE*10th International Conference on Information Technology (ICIT 2007)*, December 2007, pp. 85-90,
7. P. F. Murakami, M.R. Turner, A.K. van den Berg, and P.G. Schaberg, “An instructional guide for leaf color analysis using digital imaging software”, *Gen. Tech. Rep. NE-327. Newtown Square, PA: US Department of Agriculture, Forest Service, Northeastern Research Station, vol 33*, 2005, p, 327.
8. L.A. Van Joshua, and P.Z. Boreta, “Android-based image processing application for rice nitrogen management”, *A Thesis Report*, 2012.
9. S. Pongnumkul, P. Chaovalit, and N. Surasvadi, N.,”Applications of smartphone-based sensors in agriculture: a systematic review of research”. *Journal of Sensors*, 2015.
10. V. Patodkar, S. Simant, S. Sharma, C. Shah, and S. Godse, ”E-Agro android application (Integrated farming management systems for sustainable development of farmers)”, *International Journal of Engineering Research and General Science*, vol. *3*, no 1, 2015.
11. Y. Sanjana, A. Sivasamy, and S. Jayanth. "Plant disease detection using image processing techniques." *International Journal of Innovative Research in Science, Engineering and Technology*, vol 4, no. 6, 2015, pp. 295-301.
12. R. Subban and M. Richa. "Rule-based face detection in color images using normalized RGB color space—A comparative study." In *2012 IEEE International Conference on Computational Intelligence and Computing Research*, 2012, pp. 1-5.
13. G. D. Finlayson and G.Y. Tian. “Color Normalization for Color Object Recognition.” *International Journal of Pattern Recognition and Artificial Intelligence*, vol. 13, no. 08, 1999, pp. 1271-1285.
14. R. Fisher, S. Perkins, A. Walker, and E. Wolfart. "Hypermedia image processing reference." *England: John Wiley & Sons Ltd.*, 1996, pp. 45-46.

AUTHORS PROFILE

Geraldin B. Dela Cruz is currently with the Tarlac Agricultural University, Camiling, Tarlac, Philippines. He completed his Doctor in Information Technology degree at the TIP-QC, Philippines in 2015. He also finished his Masters of Science in Information Technology at Hannam University in South Korea in 2003 and his Bachelor of Science in Computer Science in 1997 ay Colegio de Dagupan, Philippines. He has published several research papers in the field of information technology. He is interested in the following research fields: smart/intelligent systems, data mining/science, algorithms, automation, and machine vision. Dr. Dela Cruz became a member of IACSIT and IAENG and its societies in 2013.

AJAB

Asian J Agric & Biol. 2019;7(4):564-572.

Original Article

Molecular characterization of lactoferrin gene as genetic marker to subclinical mastitis in water buffaloes (*Bubalus bubalis*)

Yancy Ogdamin Waminal[1,2], Gabriel Alexis San Perdo Tubalinal[3,4], Claro Niegos Mingala[2,3,4*]

[1]Department of Animal Science, College of Agriculture, Tarlac Agricultural University, Camiling 2306, Tarlac, Philippines

[2]Department of Animal Science, College of Agriculture, Central Luzon State University, Science City of Munoz 3120, Nueva Ecija, Philippines

[3]College of Veterinary Science and Medicine, Central Luzon State University, Science City of Munoz 3120, Nueva Ecija, Philippines

[4]Biosafety and Environment Section, Philippine Carabao Center, Science City of Munoz 3120, Nueva Ecija, Philippines

Received:
May 22, 2019

Accepted:
October 05, 2019

Published:
December 31, 2019

**Corresponding author email:*
cnmingala@hotmail.com

Abstract

The study characterized the lactoferrin (Lf) gene in different water buffalo breeds and determined its association with subclinical mastitis (SCM). A total of seventy five (75) and five (5) milk and blood samples, respectively were collected for the conduct of this study. Amplified Lf gene demonstrated a 2224 base pairs (bp) molecular weight. Nucleotide and amino acid sequence of Lf gene of riverine- and swamp-type water buffaloes revealed 98.83% and 98.29% identity, respectively with that of *Bubalus bubalis* Lf gene in the GenBank. Phylogenetic studies showed that Lf genes of both types of water buffaloes grouped with Lf gene of water buffalo sequences registered in the GenBank. Three water buffalo genotypes were documented using the restriction enzymes *AluI* and *HaeIII*. The *AluI* can produce three genotypes (AA, AB, BB) resulting in three cuts of 561, 217 and 123 bp. Using *HaeIII*, three restriction patterns were observed producing three genotypes as well with four fragment sizes of 561, 318, 112, and 70 bp. Based on the statistical analysis, no direct association has been established between the Lf genes of water buffalo with SCM using *AluI* and *HaeIII* restriction enzymes although a higher number of animals with genotype BB belongs to those with SCM group. The results of the study merit for further studies regarding the BB genotype of Lf gene as a possible resistance gene marker for SCM in water buffaloes.

Keywords: *Bubalus bubalis*, Lactoferrin, Subclinical mastitis

How to cite this:

Waminal YO, Tubalinal GASP and Mingala CN, 2019. Molecular characterization of lactoferrin gene as genetic marker to subclinical mastitis in water buffaloes (*Bubalus bubalis*). Asian J. Agric. Biol. 7(4):564-572.

This is an Open Access article distributed under the terms of the Creative Commons Attribution 3.0 License. (https://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Domesticated water buffaloes (*Bubalus bubalis*) are major milk-producing animals in several countries and contribute significantly to global milk production (FAO, 2016). Unlike dairy cows, water buffaloes are resistant to most dairy diseases even with stressful environments and settings of low feeding (Sahin et al., 2016a; Sahin et al., 2016b; Sahin et al., 2017). Distinct anatomical features, such as long narrow teat canal,

teat skin less prone to chapping and sores, thicker epithelium and keratin layer and tighter sphincter of streak canal, and absent milk cisterns may be the reason for this relative resistance (Thomas et al., 2004). However, buffaloes that are managed intensively, mastitis begins to occur even with these distinct anatomical features (Preethirani et al., 2015). The impact of mastitis is comparable to that of dairy cows involving production losses, culling and treatment costs, and decreasing animal health and welfare (Hogeveen and Van der Voort, 2017; Fagiolo and Lai, 2006; Halasa et al., 2007).

Mastitis is an economically significant infectious disease of dairy animals. Losses are due to decrease quantity and quality of milk, heightened by medicine and labor cost. A retrospective study on the prevalence of subclinical mastitis (SCM) conducted in the Philippines from September 2006 to June 2009 showed 42.76% with recurrence of 75% in water buffaloes (Salvador et al., 2012). In reducing economic losses, lowering the cases of SCM in farms is a vital strategy. To understand the mechanism of the susceptibility of animals to mastitis, genetic traits for milk production should be considered. Thus, genetic markers in the selection of animals for breeding has also been considered (Gholizadeh et al., 2008).

Lactoferrin (Lf), also known as "red protein from milk", is an iron-binding protein that can be found in most bodily fluids. Lf is released by neutrophils and inflamed tissues and has relationship with innate immunity due to its direct antimicrobial property as it limits bacterial proliferation and adhesion in the process of killing the microbes (Walker, 2010; Van Der Strate et al., 2001; Valenti and Antonini, 2005; Legrand et al., 2005; Legrand et al., 2008; Siqueiros-Cendon et al., 2014).

Information about Lf gene in water buffaloes (*Bubalus bubalis*) is limited. Genetic characterization of the gene would augment the ability to understand its role in the occurrence and onset of mastitis (Pawlik et al., 2009). The potential of Lf gene as a genetic marker for mastitis resistance in dairy animals needs to be elucidated to validate its role (Shimazaki and Kawai, 2017).

Identification of nucleotide polymorphism as a marker-associated resistance or tolerance to mastitis may be used as a tool in improving the performance of water buffaloes. Molecular characterization and genetic diversity of Lf gene of cattle have been associated with different production trait and diseases, thus, have been incorporated for selection criteria and production (Sharma et al., 2015). With this, it is rational to study the relationship of Lf gene with resistance to mastitis for water buffaloes.

The objective of the study is to characterize and identify Lf gene as a genetic marker for SCM resistance in water buffaloes by molecular analysis and association of nucleotide polymorphisms with the presence of SCM.

Material and Methods

Sample collection

A total of seventy-five (75) milk samples from riverine type water buffaloes on the 2nd month of lactation and five (5) blood samples from swamp-type water buffaloes were collected for the study. Fifty (50) and twenty-five (25) samples were non-mastitic and mastitic, respectively. There were no alterations made on the overall management of the water buffaloes included in the study.

The test animals were manually milked from the udder and pooled to collect 30 mL of milk sample for RNA extraction. The milk was collected in a 50 mL conical tube and was placed in a cooler with ice transported to the laboratory and processed on the same day of collection. For samples that were not processed on the same day were stored in a freezer (-20 °C) until used.

For the five swamp-type water buffaloes, RNA was extracted from blood as these animals were not lactating. Five mL of blood was collected from the jugular veins of the animals using EDTA tube. Samples were processed on the same day of collection.

RNA extraction

RNA extraction from milk and blood samples from the water buffaloes was done following the Promega protocol with modifications. For milk, 30 mL of raw milk was centrifuged at 4,000 rpm for 20 mins. After centrifugation, the fat layer and the supernatant were discarded, and afterward, the pellet was suspended in 1 mL PBS (phosphate-buffer-saline, a pH of 7.4). One mL of PBS was mixed again with the pellet and was centrifuged for 14,000 rpm for 1 min. This process was repeated twice and the supernatant was removed to collect the pellet.

For blood, 500 µL of the buffy coat was aspired after centrifugation at 4,000 rpm for 5 min and transferred into the 2 mL microtube, and then added with 2-3 volumes of 1 ml of 0.14 NH_4Cl. The mixture was mixed by vortex and centrifuged at 14,000 rpm for 1 min. The supernatant was removed and the process

was repeated twice to collect the pellet.
The pellet from the milk and the blood was added with 1 mL cell lysis solution and was subjected to vortex to mix the pellet and cell lysis. After this, it was centrifuged to 14,000 rpm in 1 min. The supernatant was discarded and 500 µL of nuclei lysis and 300 µL protein precipitation solution was added.
After discarding the supernatant, isolated cells were dissolved with 1 mL Trizol reagent and incubated for 10 min at room temperature. The solution was decanted and pellets were washed by 1 ml 75% ethanol and centrifuged for 5 min at 8,000 rpm at 4°C. Pellets were dried and reconstituted with 30 µL diethyl pyrocarbonate (DEPC) treated water.

Reverse transcription PCR (RT- PCR)
Complementary DNA (cDNA) was done using the cDNA Synthesis Kit (Takara Bio, Inc, Japan). One µL of oligo dT random primers were mixed with 1 µL dNTP, 5 µL of RNAse free water and 3 µL of RNA template. The solution was incubated for 5 min at 65°C before running for PCR. The prepared RNA primer mix was mixed with 4 µL 5X buffer, 0.5 µL RNAse inhibitor, 1 µL reverse transcriptase, and 4.3 µL RNAse free water. This was subjected to PCR run for segment 1, 10 min at 30°C; segment 2, 45 min at 50°C; and segment 3, 5 min at 95°C. The synthesized cDNA was tested for *β-actin* gene amplification to ensure cDNA synthesis protocol.

PCR primers
Three primer sets were used to amplify the Lf gene (Table 1). These primers were designed using the Primer3 server (http://biotools.umassmed.edu/bioapps/primer3_www.cgi) and Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) using the bubaline sequence (Accession number: JF825526) from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/).
The designed primers were analyzed for self-annealing and loop properties using Oligo analyzer software (https://sg.idtdna.com/calc/analyzer). Primers forming hairpin loops, self-annealing, dimers, cross dimer, and repeats were avoided as much as possible. Suitable primers were checked using BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to ensure that it amplifies the Lf gene.

Table-1: Nucleotide and amino acid sequence percentage similarity of Lf gene in ruminant species regarding swamp-type and riverine-type water buffaloes.

Primers	Nucleotide Sequence	Expected Product Size
Primer 1 - F	TAGCCATGAAGCTCTTC	835 bp
Primer 1- R	GAGTACGGACAACACCGGGC	
Primer 2 - F	GCTTCTCTGCCTAAACAACAC	1008 bp
Primer 2 - R	GTCTCAGCACACAATCTAGG	
Primer 3 - F	AACATCCCCCATGGGCCTG	694 bp
Primer 3 - R	TTTCTTCGATCGGACGGA	

Gene amplification
All PCR assays were performed in 20 µL reaction volume containing 2 µL of genomic DNA template, 10 pmol of each primer and PCR master mix. The amplification of the Lf gene was carried out in a thermocycler (SimpliAmp, Thermofisher) under optimized conditions. Briefly, initial denaturation at 94˚C for 4 min, followed by 40 cycles consisting of denaturation at 94˚C for 30 sec, annealing at 57˚C for 1 min and extension at 72˚C for 45 sec, with a final extension at 72˚C for 4 min.
After amplification, 1 µL of the PCR product was electrophoresed on 1.5 % agarose gel containing 1X TAE buffer at 70 volts for 30 min and visualized under ultraviolet light using UV transillumination advanced imaging system. A 1kb plus DNA ladder (Invitrogen, CA, USA) was used as a ladder to determine the sizes of the PCR products.

Nucleotide sequencing and phylogenetic analysis
Lf products were submitted for sequencing at the Philippine Genome Center. DNA sequences were assembled using Mega 7 software. The forward and reverse sequences for each primer were assembled to form contigs of the respective regions. The gene sequences were compared with the Lf mRNA sequences to annotate different exonic regions putatively to identify SNPs in respective regions. The contiguous Lf gene nucleotide sequence was subjected to Basic Local Alignment Search Tool (BLAST) at the NCBI database to determine the sequence similarity with the corresponding regions of other species.
Nucleotide sequences were aligned and translated to the amino acid sequence using the MEGA 7 software.

The phylogenetic tree was constructed using the neighbor-joining method. Confidence in the groups was estimated by a bootstrap of data using 1000 replications. Phylogenetic trees were constructed using MEGA 7 software (Tamura et al., 2011).

RFLP analysis

The Lf PCR new set of primers sequence in water buffaloes (F-5' ATCCTTCGCCCGTTCTTGAG 3' and R-3' CGCCGAATCTACTTTTGAGG 5') was designed to amplify the ligand-binding region in exon 2, 3 and 4 of Lf gene in water buffaloes in which the putative area is located with an amplicon size of 561 bp.

Restriction enzymes *AluI and HaeIII* were used based on the suggested enzymes by Sequence Manipulation Suite: Restriction map (http://www.bioinformatics.org/sms2/rest_map.html) (Stothard, 2000).

Restriction fragment length polymorphism (RFLP) was conducted by preparing the reaction mixture composed of 7 µl of PCR product, 5.15 µl of sddH_2O, 0.35 RFLP buffer and 0.075 µL for each enzyme. Samples were incubated at 37°C for 4 hr. Restriction fragments were resolved on 2% agarose gel in horizontal electrophoresis. The restriction-digested gene fragments were visualized on a UV transilluminator (FlourChem E by ProteinSimple ™) and photographed.

Association of Lf gene to SCM

The amplified fragment of the Lf gene from the milk of water buffaloes showed different genotypes based on the exhibited banding patterns from *AluI* and *HaeIII* restriction enzymes. The different genotypes were associated based on the result of the CMT test of each of the milk samples. A total of 62 samples from water buffaloes were used in studies that associated expression of Lf gene and the occurrence of SCM.

CMT analysis

CMT was used as an indirect method of measuring Somatic Cell Count (SCC) as the indicator of intramammary infection as it has the advantage of being quick, cheap, and simple "animal side" test.

All milk samples collected were subjected to CMT. A small amount of milk (approximately 5 mL) was put into the paddle. The paddle was tilted almost vertically so that only 2 to 1 teaspoon of milk remains in each cup. An equal amount of CMT reagent was added to the milk and swirled for about 15 seconds. Observation of the reaction was done immediately to see if there is any thickening of the milk. The thicker the mixture, the higher the SCC. In goats and water buffaloes, the reaction scores are: N (negative) with no reaction and with estimated SCC of below 200,000 cells/mL; T (trace) with slight slime, tends to disappear with continued swirling with 150,000 to 500,000 cells/mL; 1 when there is distinct slime but without gel with 400,000 to 1,500,000 cells/mL; 2 when there are immediate gel formation and moves as a mass during swirling with 800,000 to 5,000,000 cells/mL; and 3 when gel develops a convex surface and adhere to the bottom of the cup with cell count estimated above 5,000,000 cells/mL (Rahman et al., 2010).

CMT scores for all animal subjects were classified as non-mastitic if the CMT score result is 1 or lower and mastitic if the CMT score result is 2 or higher.

Statistical analysis

Univariate analysis on the possible association between the genotypic frequency and the occurrence of SCM was examined using Chi-square (X^2) test analysis for the goodness of fit (Petrie and Watson, 2006).

$X^2 = E\,(O-E)^{2E}$

Where,

O= Observed Frequencies

E= Expected Frequencies

Results and Discussion

A study on the detection and molecular characterization of Lf gene in blood and milk samples of water buffaloes (riverine- and swamp-types) was undertaken. Out of all samples collected, one sample of each type of buffalo was sent for sequencing.

Figure 1 shows that the target primers 1, 2, and 3 were amplified and generated an amplicon size of 835 bp, 1008 bp, and 694 bp, respectively.

Sequence analysis of Lf Genes

In water buffaloes, swamp-type Lf mRNA sequence resulted in an average of 2224 bp (LC425118.1) while 2226 bp (LC425117.1) in riverine-type water buffalo.

Table-2: Nucleotide and amino acid sequence percentage similarity of Lf gene in ruminant species regarding swamp-type and riverine-type water buffaloes.

SPECIES (Accession Number)	NUCLEOTIDE SEQUENCE		AMINO ACID SEQUENCE	
	Swamp (LC425118.1)	Riverine (LC425117.1)	Swamp	Riverine
B. bubalis (JF825526.1)	98.29%	98.83%	97%	98%
B. Taurus (FJ589071.1)	95.42%	95.98%	96%	96%
B. indicus (GU059864.1)	95.28%	95.84%	95%	96%
B. grunniens (DQ387455.1)	95.35%	95.91%	95%	96%
C. hircus (U53857.1)	90.69%	91.19%	91%	92%
O. aries (NM_001024862.1)	92.66%	93.23%	93%	94%
E. caballus (NM_001163974.1)	74.06%	74.37%	75%	75%
S. scrofa (M81327.1)	73.69%	74.01%	74%	73%
H. sapiens (U076343)	70.76 %	71.98 %	69%	71 %

The riverine Lf gene nucleotide sequence was aligned with other ruminants' sequence of Lf gene using the database from GenBank. The 14 aligned sequences showed an average of 2127 identical pairs, 31 transitional pairs, and 18 tranversional pairs. Between riverine-type, swamp-type water buffalo, and *B. bubalis* (JF825526.1) nucleotide sequence, the average identical pairs were 2221 bp with 3 transversional pairs. This showed that there is a high similarity of sequence between the two types of water buffaloes

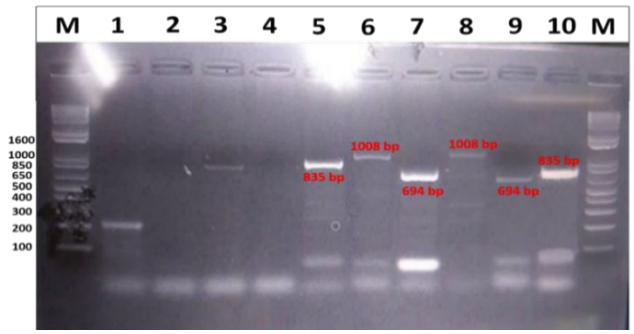


Figure-1: Amplification of Lf gene of swamp-type and riverine-type water buffaloes using primers 1, 2, and 3. Lane M (1kb plus ladder); Lanes 1-4 unamplified PCR products. Lane 5 and 10 (Primer 1); Lane 6 and 8 (Primer 2); Lane 7 and 9 (Primer 3)

The nucleotide BLAST of Lf coding sequence of the riverine and swamp samples revealed a high percentage of similarity between the query sequence and other species of Lf sequence available in the NCBI database (Table 2). Riverine-type Lf gene nucleotide sequence was 98.83% similarity with that of *B. bubalis* (JF825526.1), 95.98% similarity with *B. taurus* (FJ589071.1), 95.91% with *B. grunniens* (DQ387455.1) and 95.84% *B. indicus* (GU059864.1). The lower similarity was observed with small ruminant species such as *O. aries* (NM_001024862.), *C. hircus* (U53857.1) and other species such as *E. caballus* ((NM_001163974.1), *S. scrofa* (M81327.1), and *H. sapiens* (U076343).

Similarly, the protein BLAST result presented a high similarity of translated amino acid sequences of riverine-type Lf to *B. bubalis* (JF825526.1). A lower similarity in water buffaloes Lf amino acid sequence was observed with *B. taurus*, *B. grunniens*, *C. hircus* and *O. aries*, respectively.

Phylogenetic analysis

The maximum likelihood NJ algorithm with 1000 bootstrap resampling revealed the clustering of water buffaloes. Swamp-type water buffalo Lf gene clustered together under a single clade with *B. bubalis* sequence from GenBank being related to swamp-type water buffaloes but more descendants of riverine-type water buffaloes. *B. taurus* and *B. grunniens* can also be descendants of the bubaline Lf while *C. hircus* and *O. aries* are distant relatives.

However, the phylogenetic tree analysis (Figure 2) showed a similar evolutionary origin of riverine-type and swamp-type water buffaloes in consonance with *B. bubalis* Lf generated from the Genbank. This, therefore, could indicate minimal variation on the susceptibility or resistance to diseases.

Polymorphism analysis

The coding sequences of Lf gene in water buffaloes were analyzed for differences in nucleotides and functional amino acid substitution. Furthermore,

restriction enzyme capable of digesting the sequence to identify the nucleotide polymorphism and distinguish the genotype has been identified and results are herein presented.

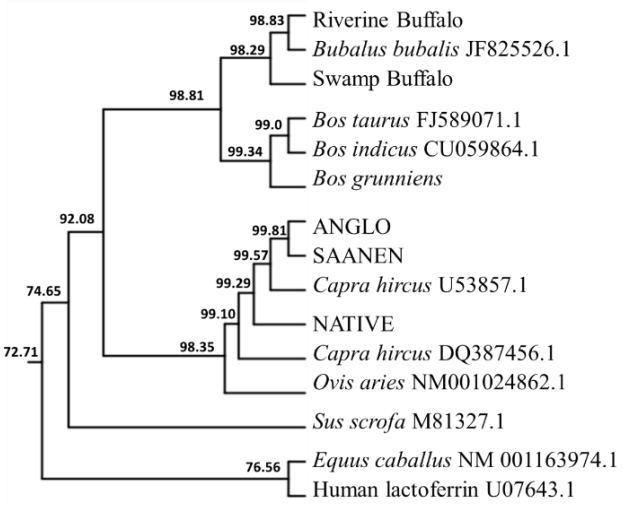


Figure-2: Phylogenetic tree showing the relationship of Lf gene between water buffaloes, goats, other ruminants' and human

To develop rapid recognition of polymorphic site of Lf gene, restriction enzymes *AluI* and *HaeIII* were used to digest 561 bp that was amplified by the primer 2. This 561 bp segment covered exons 2 and 3 of the coding region. SMS Restriction Digest (http://www.bioinformatics.org) application was used for the four samples with a complete Lf gene nucleotide sequence.

The availability of these restriction patterns on extracted Lf gene was examined by using a PCR product that was subjected to *AluI* and *HaeIII* digestion and electrophoresed. Results showed a restriction pattern that was almost the same base on the conceptualized expected pattern. From the 2224 bp nucleotide sequence, it revealed three-nucleotide polymorphism in riverine-type at locus 514, 857, and 1039 (Table 3) with two amino acid substitutions and in swamp-type water buffalo there are four nucleotide polymorphism in the Lf coding sequence at locus 315, 817, 1039 (Table 4), and 1488 with two amino acid substitutions.

The nucleotide polymorphism at locus 857of two types of water buffalo Lf gene sequences resulted in a functional amino acid substitution from threonine to isoleucine. However, there is no amino acid substitution at locus 1039, although there are changes in their nucleotides.

Lee et al. (1997), Martin-Burriel et al. (1997), Li and Chen (1999) and Kaminski et al. (2006) reported that polymorphisms in Lf gene reportedly occur in the coding and regulatory regions and polymorphisms are encoded in exons and introns.

Table-3: Nucleotide polymorphism in Lf gene of riverine-type water buffalo and corresponding sites of amino acid substitution

Base location		Nucleotide polymorphism		Amino acid location	Amino acid substitution	
1	514	C	T	202	L (Leucine)	P (Phenylalanine)
2	857	C	T	286	T (Threonine)	I (Isoleucine)
3	1039	C	T	347	Same	

Table-4: Nucleotide polymorphism in Lf gene of swamp-type water buffaloes and corresponding sites of amino acid substitution

Base location		Nucleotide polymorphism		Amino acid location	Amino acid substitution	
1	315	G	C	105	E (Glutamic acid)	D (Aspartic acid)
2	857	C	T	286	T (Threonine)	I (Isoleucine)
3	1039	C	T	347	Same	
4	1488	C	T	496	Same	

The results of this study corroborated with the result of previous Lf gene studies. In addition, Seyfert et al. (1996) mentioned that the number of exons in the gene and the number of amino acids encoded by 15 of the 17 exons are identical among them. The major differences lie in exons 2, where bovine and other animals' Lf contains one or two amino acids less than human Lf. However, Rupp and Boichard (2003) reported that there is still no strict association found between known Lf gene polymorphisms and mastitis susceptibility.

Restriction enzyme *AluI* cuts AG/CT nucleotide sequence was used to examine polymorphisms in the nucleotide sequence of Lf gene via banding patterns and were compared to the Lf gene sequences of water buffaloes in the GenBank. It was revealed that *AluI* can produce three genotypes at the nucleotide sequence of the 561 bp amplicon. Genotype AA produced two bands of 561 and 123 bp size; one cut was also produced in genotype AB of 217 and 123 bp, and genotype BB that produced two cuts resulting in 561, 123, and 217 bp amplicon sizes (Figure 3).

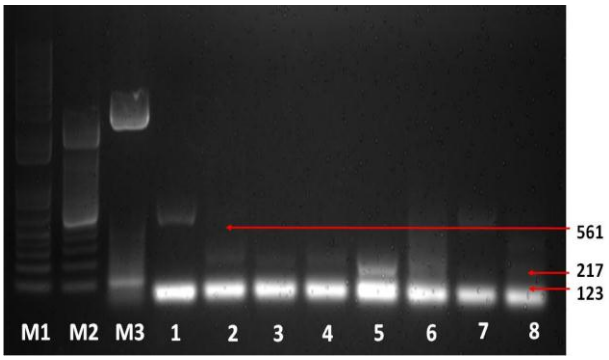


Figure-3: Restriction patterns of Bubaline Lf gene PCR product using *AluI* in 2% agarose gel. M1. 1000 bp ladder, M2. 100 bp ladder. M3. 25 bp ladder, Lanes 1 and 7 genotype AA (fragment size: 561, 123 bp), Lanes 2, 3, 4 and 5-genotype AB (217, 123 bp), Lanes 6 and 8-genotype BB (size: 561, 217, 123, bp).

Figure 4 shows the result of the digestion of water buffalo Lf gene PCR products using *HaeIII* enzyme. From the conceptualization four restriction patterns from nucleotide sequence were observed, genotype AA had one cut with a fragments sizes of 561 and 70 bp; genotype AB with three cuts of 561, 318, 112, and 70 bp, and genotype BB with two cuts of 318, 112, and 70 bp.

No recent studies supported in the three different genotypes found in the riverine water buffalo Lf gene using *HaeIII* and *AluI* restriction enzyme, hence this study will be the baseline for other researchers in identifying mastitic resistance genotypes.

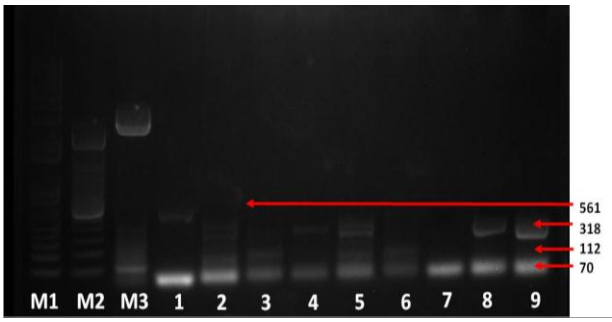


Figure-4: Restriction patterns of Bubaline Lf gene PCR products using *HaeIII* in 2% agarose gel. M1. 1000 bp + ladder, M2 100 bp ladder. M3 25 bp ladder, Lane 1 and 7, genotype AA (fragment size: 561, 70 bp), Lanes 2, 3, 5 and 6, genotype AB (fragment size: 561, 318, 112 and 70 bp), Lanes 4, 8 and 9, genotype BB (fragment size: 318, 112 and 70 bp)

Association of Lf Gene to SCM

From the total of 75 milk samples, only 62 milk samples were used in for RFLP analysis.

The 561 bp putative ligand-binding domain was targeted in this segment of the study. Table 5 shows the percentage frequency of Lf *AluI*-based genotype in non-mastitic and sub-clinically mastitic riverine water buffalo.

Table-5: Percentage frequency of Lf *AluI*-based genotypes in non-mastitic and SMC riverine-type water buffaloes

Genotype	Category of animal				Total	
	Non- mastitic		SMC			
	%	(n)	%	(n)	%	(n)
AA	66.67	14	33.33	7	34.4	21
AB	57.14	12	42.86	9	34.4	21
BB	52.63	10	47.37	9	32.2	19
Total	59.02	(36)	40.98	(25)	100	61

* bp = base pair

From a total of 61 milk samples tested, 40.98% (n=25) were considered sub-clinically mastitic, while 59.02 % (n=36) were non-mastitic. Genotype AA was found to be more frequent in non-mastitic animals, although Chi-square analysis did not show any significant difference with that of genotype. Furthermore, Statistical analysis of the result could not provide concrete evidence that resistance to SCM was due to the specific genotype.

The Lf gene in water buffaloes has a bactericidal and bacteriostatic activity that could lessen the bacterial population in the milk through phagocytic killing (Valenti and Antonini, 2005; Legrand et al., 2008). This may not be enough to sequester the microbial flora in the mammary gland of the animals. Underlying factors such as climate, housing system, type of bedding, and rainfall and wetness in the vicinity of dairy premises interact to influence the degree of exposure of teat and tend to increase mastitis pathogens that cause SCM (McEwen and Cooper, 1947).

Table 6 shows the percentage frequency of Lf *HaeIII*-based genotype in non-mastitic and SCM riverine water buffalo. From a total of 62 milk samples tested, 35.48% (n=22) were considered SCM, while 64.52% (n=40) were not mastitic. Genotype AA and AB were found to be more frequent in non-mastitic animals, however, Chi-square analysis did not show any significant difference with these genotypes.

The inflammation of the mammary gland induces epithelial cells to secrete Lf. The regulatory region of this gene is useful in the expression of the other antimicrobial proteins with the more potent anti-inflammatory action than Lf itself. Furthermore, Lf cannot work alone thus, animal genetics must coincide with good management practices to prevent or minimize the possible occurrence of SCM in a dairy herd.

Both digestion enzyme used may not have shown a significant association of the investigated genotype, but the presence of extensive polymorphic nucleotide in Lf gene can still be investigated using other restriction enzymes that could target the said polymorphic loci.

Table-6: Percentage frequency of Lf *HaeIII*-based genotype in non- mastitic and sub-clinically mastitic riverine-type water buffaloes

Genotype (fragment sizes)	Category of animal				Total	
	Non- mastitic		Sub-clinically mastitic			
	%	(n)	%	(n)	%	(n)
AA	28.57	15	71.43	6	33.87	21
AB	70.00	14	30.00	6	32.26	20
BB	47.62	10	52.38	11	33.87	21
Total	35.48	(22)	64.52	(40)	100	62

* bp = base pair

Conclusion

The whole mRNA coding sequence of water buffaloes had an average molecular weight of 2224 bp. Both nucleotide and translated amino acid sequences of Lf in water buffaloes were highly similar (99%) and phylogenetic analysis found an evolutionary relationship with water buffaloes from NCBI GenBank. The Lf gene of riverine water buffaloes established three different genotypes (AA, AB, and BB) using restriction enzymes *AluI* and *HaeIII*. No direct relationship existed between Lf gene and SCM. These initial findings are applicable in the field of immunity and disease resistance. The incidence of association between restriction sites and clinical parameter converts PCR-RFLP into a powerful tool in relating specific amino acid substitution at a critical position to possible disease resistance. Moreover, the selection of disease resistance genes will provide potential avenues for improving the health status of the animal and increasing productivity.

Lactoferrin is a very polymorphic gene that merits further investigation and its significance on disease resistance and susceptibility. Polymorphism of this gene may be useful as a selection marker for resistance against SCM. The result of this study may not statistically prove that lactoferrin can be a marker for SCM. However, the study also points out that most of the water buffaloes with SCM fall under the BB genotype.

Acknowledgment

The authors would also like to thank the Philippine Carabao Center for their support in this study. Moreover, due credits to the Biosafety and Environment Section staff for their technical assistance.

Disclaimer: None
Conflict of Interest: None
Source of Funding: None.

References

Fagiolo A and Lai O, 2006. Mastitis in buffalo. Italian Journal of Animal Science. 6: 200-206. https://doi.org/10.4081/ijas.2007.s2.200

FAO, 2016. FAO report. http://www.fao.org/agriculture/dairy-gateway/milk-production/dairy-animals/water-buffaloes/en/#. V-8-c_REfK8. Retrieved on July 1, 2019.

Gholizadeh G, Mianji GR, and Zadeh HS, 2008. Potential Use of Molecular makers in the Genetic Improvement of Livestock. Asian J. Anim. Vet. Adv. 3: 120-128.

Halasa T, Huijps K, Osteras O and Hogeveen H, 2007. Economic effect of bovine mastitis and mastitis management: A review. Vet Q. 29: 18-31. https://doi.org/10/1080/01652176.2007.9695224

Hogeveen H and Van der Voort M, 2017. Assessing the economic impact of an endemic disease: the case of mastitis. Rev. Sci. Tech. 36: 217-226. https://doi.org/10.20506/rst. 36.1.2623

Kaminski S, Olenski K, Brym P, Malewski T and Sazanov AA, 2006. Single nucleotide polymorphism in the promoter region of the lactoferrin gene and its associations with milk performance traits in Polish Holstein-Friesian cows. Russian J. Genet. 42: 924-927.

Lee TH, Shimazaki K, Yu SL, Nam MS, Kim SJ, Lee KK and Yu DY, 1997. Polymorphic sequence of Korean native goat lactoferrin exhibiting greater antibacterial activity. Anim. Genet. 28: 367–369.

Legrand D, Elass E, Carpentier M and Mazurier J, 2005.

Lactoferrin: a modulator of immune and inflammatory responses. Cell. Mol. Life. Sci. 62. 2549-2559

Legrand D, Pierce A, Elass E, Carpentier M, Mariller C and Mazurier J, 2008. Lactoferrin structure and functions. Adv. Exp. Med. Biol. 606: 163-94.

Li SH and Chen YH, 1999. Various forms of mouse lactoferrins: purification and characterization. J. Chromatogr. B Biomed. Sci. Appl. 726: 45–52.

Martin-Burriel I, Osta R, Baredse W and Zaragoza P, 1997. New polymorphism and linkage mapping of the bovine lactotransferrin gene. Mammal. Genom. 8: 704-705.

McEwen AD and Cooper MB, 1947. Bovine mastitis. Vet. Rec. 59(48): 655-664.

Pawlik A, Sender G and Korwin-Kossakowska. 2009. Bovine lactoferrin gene polymorphism and expression in relation to mastitis resistance - A review. Anim. Sci. Pap. Rep. 27(4): 263-271.

Petrie A and Watson P, 2006. Statistics for Veterinary and Animal Science. 2nd Edition. Wiley-Blackwell, 312 p.

Preethirani PL, Isloor S, Sundareshan S, Nuthanalakshmi V, Deepthikira K and Sinha AY 2015. Isolation, Biochemical, and Molecular Identification, and In-Vitro Antimicrobial Resistance Patterns of Bacterial Isolated from Bubaline Subclinical Mastitis in South India. PLOS One. 10: e0142717.

Rahman MM, Islam MR, Uddin MB and Aktaruzzaman M, 2010. Prevalence of subclinical mastitis in dairy cows reared in Sylhet District of Bangladesh. Int. J. Biol. Res. 1(2): 23–28.

Rupp R and Boichard D, 2003. Genetics of resistance to mastitis in dairy cattle. Vet. Res. 34: 671-688.

Sahin A, Yildirim A and Ulutas Z, 2016a. Changes in some physico-chemical content of Anatolian Buffalo milk according to the some environmental factors. Buffalo Bull. 35: 573-585.

Sahin A, Yildirim A and Ulutas Z, 2016b. Effect of Various Environmental Factors and Management Practices on Somatic Cell Count in the Raw Milk of Anatolian Buffaloese. Pak. J. Zool. 48: 325-332.

Sahin A, Yildirim A and Ulutas Z, 2017. The effects of stage of lactation, parity and calving season on somatic cell counts in Anatolian Water Buffaloes. Indian J. Anim. Sci. 51: 35-39.

Salvador RT, Beltran JM, Abes NS, Gutierrez CA and Mingala CN, 2012. Prevalence and risk factors of subclinical mastitis test in water buffaloes (*Bubalus bubalis*) in Nueva Ecija, Philippines. J. Dairy Sci. 95: 1363-1366.

Seyfert HM, Henke M, Interthal H, Klussmann U, Koczan D, Natour S, Pusch W, Senft B, Steinhoff UM, Tuckoricz A and Hobom G, 1996. Defining candidate genes for mastitis resistance in cattle: the role of lactoferrin and lysozyme. J. Anim. Breed. Genet. 113: 269-276.

Sharma R, Chakraborty D and Gupta P, 2015. Bovine lactoferrin and its functions in animals – A review. Agric. Rev. 36(4): 321-326.

Shimazaki KI and Kawai K, 2017. Advaces in lactoferrin research concerning bovine mastitis. Biochem. Cell. Biol. 95(1): 69-75. Doi: 10.1130/bcb-2016-0044

Siqueiros-Cendon T, Arevalo-Gallegos S, Iglesias-Figueroa BF, Gracia-Montoya IA, Salazar-Martinez J and Rascon-Cruz Q, 2014. Immunomodulatory effects of lactoferrin. Acta. Pharmacol. Sin. 35(5): 557-566. DOI: 10.1038/aps.2013.200

Stothard P, 2000. The Sequence Manipulation Suite: JavaScript programs for analyzing and formatting protein and DNA sequences. Biotechniques. 28: 1102-1104.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M and Kumar S, 2011. Mega 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol. Biol. Evol. 28(10): 2731-2739.

Thomas CS, Svennersten-Sjaunja K, Bhosrekar MR and Bruckmaier RM, 2004. Mammary cisternal size, cisternal milk and milk ejection in Murrah Buffaloes. J. Dairy Res. 71: 162-168.

Valenti A and Antonini G, 2005. Lactoferrin: an important host defense against microbial and viral attack. Cell. Mol. Life. Sci. 62: 2576-87.

Van der Steate BWA, Belijaars L and Molema G, 2001. Antiviral activities of lactoferrin. Antiviral Res. 52: 225-239.

Walker A 2010. Breast milk as the gold standard for protective nutrients. J. Pediat. 156: 3-7.

Contribution of Authors

Waminal YO: Designed and conducted the study and prepared the manuscript of the draft

Tubalinal GASP: Conducted the study and prepared the manuscript draft

Mingala CN: Designed and supervised the study and prepared and approved the final version of the manuscript draft

YOGURT FORTIFIED WITH PURPLE ROSELLE EXTRACT AS PREVENTION OF DIOXIN INTOXICATION BASED ON MALONDIALDEHYDE LEVELS AND HISTOPATHOLOGY OF RATS (*RATTUS NORVEGICUS*) KIDNEY

Ani Setianingrum*, Allan Jeffrey Francia[1], Dhanti Kusumaningdyah, Edwin Widodo[2], Aldila Noviatri[3], Allan Jeffrey Francia[1] and Ajeng Erika P Haskito

Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Brawijaya University, Malang, Indonesia, 65151

Received on: 08.10.2020
Accepted on: 11.01.2021

ABSTRACT

Dioxin is one of the chemical compounds produced through the chemical industry's combustion process, can pollute the environment,and harms the health of living things. Exposure to dioxin that accumulates in the body can disrupt normal organ function, including the kidneys. This study aimed to determine the preventive effect of giving yogurt fortified with purple roselle extract on the conditions of dioxin intoxication based on the parameters of Malondialdehyde MDA levels and kidney histopathology. This study used 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which has the highest toxic potential among other dioxins congeners. TCDD was diluted in corn oil. This study used white male rats (*Rattus norvegicus*), 30 animals were divided into 5 treatment groups (1) negative control without treatment, (2) positive control was given TCDD dose of 200 ng/kg b.wt./day, (3) P1 given TCDD 200 ng/kg b.wt./day and roselle yogurt concentration of 0.5% 1 ml, (4) P2 given TCDD 200 ng/kg b.wt./day and roselle yogurt 1% concentration 1 ml, (5) P3 were given TCDD 200 ng/kg b.wt./day and roselle yogurt with a concentration of 1.5% 1 ml. The treatment was given for 12 days, orally. The parameters gathered were MDA levels and kidney histopathological changes. MDA levels were analyzed quantitatively using ANOVA continued with the Tukey test for comparison of treatment (α = 5%). Histopathological changes were analyzed descriptively. This study concludes that yogurt fortification with purple rosella extract with a concentration of 0.5%, 1.0%, and 1.5% as prevention on dioxin intoxication has not been able to decrease MDA levels of rat kidney and to prevent the histopathological damage of kidney rats.

Key words: Dioxin. MDA, kidney, purple roselle extract, yogurt

Introduction

Many activities caused environmental pollution. One of them is the combustion process of industrial waste and burning products containing chlorine. The combustion of waste has a negative effect because the compound created has a prolonged degradation rate, both in air, soil, and water. These waste compounds has long-term and short-term impacts on human, animal, and ecological health. According to Fiedler (2003) these dangerous industrial wastes are known as dioxins.

The World Health Organization data in 2012 states that dioxin levels are mostly found in eggs, poultry, milk, and fish. When dioxins enter the human or animal body, it will cause various disorders, including immune, nervous, endocrine, reproductive, and liver function changes. According to Indraningsih and Sani (2014) Trichloro dibenzo-p-dioxins (TCDDs) residues were found at a high level on beef meat in some areas in Indonesia. According to Susanti (2004), TCDD will accumulate in the tissues and have toxic effects that only appear after several years, starting with the binding of dioxins-AhR (Aryl Hydrocarbons Receptor), the chaperones dissociate, resulting in AhR translocating into the nucleus and dimerizing with ARNT (AhR nuclear translocator), leading to changes in gene transcription. Then inducing the expression of cytochrome enzymes P450 CYP-A1 and CYP-B1. These enzymes contribute to cellular reactive oxygen species (ROS). ROS formation contributes to cell damage and disease development. Exposure to dioxins shows an increase of ROS indicator in the organs of animal models, including the kidney (Sakin *et al*., 2011). To prevent the overproduction of ROS, the body requires antioxidants.

Yogurt is a fermented milk product with high antioxidant content, involving microorganisms, namely, *Lactobacillus bulgaricus* and *Streptococcus thermopillus* bacteria. Antioxidants are available from natural ingredients, especially in spices, fruits, and vegetables. A study conducted by Suharto *et al*. (2016) found that the antioxidant activity of natural ingredients is found in plant extraction. One of the plants known to have high antioxidant activity is the purple roselle. Purple roselle (*Hibiscus sabdariffa L*) has the main component of anthocyanin pigments that form flavonoids as antioxidants (Nugroho *et al*., 2018). According to Arviani *et al.* (2018). The levels of vitamin C in purple rosella were 241 mg/100 g higher than oranges (49 mg/100 g), star fruit (35 mg/100) and papaya (78 mg/100 g).

Fortification of purple rosella extract in yogurt has the organoleptic preference due to color and taste (Noviatri *et al*., 2020), and is expected to have a preventive effect of reducing the number of free radicals due to exposure to dioxins. Hence, this study was conducted to determine whether fortified yogurt with purple roselle extract could increase antioxidant activity against dioxin exposure in terms of MDA levels and kidney histopathology.

Materials and Methods

Chemicals

Dioxin compound used was TCDD (Supelco 45899), cow's milk yogurt, purple roselle extract, yogurt starter

*Corresponding author email: ani.setia@ub.ac.id; [1]Faculty of Veterinary Medicine, Tarlac Agricultural University, Camiling, Tarlac 2306, Philippines; [2]Faculty of Medicine, Brawijaya University, East Java, Indonesia; [3]Pharmacology Veterinary Laboratory, Faculty of Veterinary Medicine, Brawijaya University, Malang, Indonesia, 65151

(Yogourmet® LYO-SAN.INC) contains *L. bulgaricus*, *S. thermophilus*, and *L. acidophilus*, 10% phosphate buffered saline (PBS), paraffin block, corn oil, physiological NaCl, 2-Thiobarbituric acid (TBA), formaldehyde 10%, aquadest, and hematoxylin and eosin (HE) stain.

Yogurt fortification purple roselle extract

Dried roselle petals were ground until smooth until they became flour, then sieved using a sieve. The roselle powder was dissolved in warm water (ratio 20 g: 100 ml water). After that, the pasteurization was carried out at 63ºC - 65ºC for 30 minutes. After 30 minutes, the liquid and sediment were separated (Suharto *et al.,* 2016).

Yogurt starter was made by pasteurized 100 ml of cow's milk at a temperature of 72ºC for 15 minutes. Then, the cow's milk was cooled down to a temperature of 45ºC. After that, the yogurt starter was inoculated and homogenized. Then, it was incubated at 45ºC for 4 hours until it reached a pH of 4.4-4.5 (Mahdi *et al.,* 2016). The starter was inoculated as much as 3% of the volume of cow's milk to make cow's milk yogurt (F1) (Mahdi *et al.,* 2016). Plain yogurt homogenized using a blender and divided into 3 parts. Then the purple roselle extract was added with a concentration of 0.5%, 1%, and 1.5%. Analysis of nutrition value of yogurt fortified with purple roselle extract was done using proximate assay and antioxidant activity analyzed by DPPH modified Pinela *et al.* (2012) method.

Animals and experimental design

The study was approved by the University Institutional Animal Care and Use Committee. The animals used were rats (*Rattus norvegicus*) divided into five groups, with six (6) rats per group. Feed, and drinking water was given *ad libitum* for 7 days. Rats were housed under standard laboratory conditions (room temperature 25°C - 26°C, 12 h light-dark cycle). 2,3,7,8-Tetrachlorinedibenzo-p-dioxin (TCDD) diluted with corn oil, gave orally using a gavage every day for 12 days.

Experimental groups were: Negative control group (NC) received normal food and water; Positive control group (PC) given a dose of 200 ng/kg b.wt./day TCDD; Yogurt of cow's milk with purple rosella extract with each concentration (0.5%, 1%, 1.5%) was given to each treatment (P1, P2, and P3). Group P1 was given a dose of 200 ng/kg b.wt./day TCDD and 1 mL of 0.5% roselle yogurt; Group P2 was given a dose of 200 ng/kg b.wt./day TCDD and 1 mL of 1% roselle yogurt; and Group P3 was given a dose of 200 ng/kg b.wt./day TCDD and 1 mL of 1.5% of roselle yogurt.

Sample collection and analyses

The experimental rats (*Rattus norvegicus*) were euthanized at the end of the experiment. The abdominal cavity was incised, and kidneys were extracted immediately. The left kidney was immersed in a 10% formaldehyde solution to be used for making histopathological preparations. Then, the right kidney was wrapped in aluminum foil and stored at -20° for biochemical analyses.

Determination of (*malondealdehyde*) MDA levels using the Thiobarbituric acid (TBA) method. The kidney sample was weighed as much as 0.5 grams, crushed with a mortar, then added 200 µl of physiological NaCl. The homogeneous sample was put into a polypropylene tube and added with distilled water. Then added 100 µl of homogenized TBA, added 250 µl of 1N HCl, and homogenized again. The homogeneous mixture was added with Na-Thio 1% as much as 100 µL and centrifuged at 500 rpm for about 10 minutes. The supernatant was taken then heated in a water bath of 100°C for 20 minutes. The supernatant was then cooled at room temperature, and the absorbance value of the sample was determined using a UV-Vis spectrophotometer at its maximum wavelength (Widyaningsih *et al*., 2015).

Kidney samples were immersed into xylol concentration levels 1-3 each for 5 minutes. The dehydration was carried out by inserting the sample into stratified ethanol starting from 1-3 absolute ethanol, 95%, 90%, 80%, and 70% ethanol for 5 minutes each. The preparations were then immersed in distilled water for 5 minutes. After that, put in a hematoxylin dye for 10 minutes. The preparations were washed in running water and soaked in distilled water again to remove excess eosin. After that, the dehydration process, the preparations were put into the ethanol series, graded from 80%, 90%, and 95% to absolute ethanol 1-3. The last process, namely clearing by inserting the preparations into xylol 1 and 2, then drying. The mounting process was carried out using Etellan®. After the mounting process, the prepared slides were observed and examined under the light microscope equipped with a digital camera and the images were processed using a software and each sample was observed in five fields view. Observations were made to determine epithelial necrosis, glomerular and tubular damage.

Data analysis

Statistical analyses using One Way Analysis of Variance (ANOVA) continued with Honest Real Difference (HSD) Tukey's test (α = 5%). The results of the observation of the histopathological preparations of the kidney were analyzed descriptively.

Results and Discussion

Table 1 shows the result on yogurt fortified with purple roselle extract nutritional and antioxidant activity analysis. Antioxidant activity assay for yogurt fortified with purple roselle extract IC_{50} result was increasing as the concentration of the purple roselle extract increase.

Table 1:Analysis of yogurt fortified with different concentration of purple roselle extract

Sample	Test	0.5%	1.0%	1.5%
Yogurt fortified with purple roselle extract	Protein (%)	2.26	1.98	1.99
	Fat (%)	3.32	3.92	3.75
	Water (%)	89.21	89.17	89.25
	Ash (%)	0.75	0.74	0.74
	Carbohydrate (%)	4.46	4.19	4.27
	Antioxidant IC_{50} (mg/mL)	181.10	140.68	137.70

Table 2 shows the MDA level assay of all treatment groups. MDA level on all treatment groups showed a significant increase compared to negative control group.

Fig. 1 shows the kidney histopathological changes on each group with HE staining under light microscope with 400x magnification. The histopathology of rat kidney showed differences in each treatment group according to the changes in glomerulus, Bowman's space, and kidney tubular.

Table 2: MDA level of rat's kidney of all groups in the study

Groups	Kidney MDA level (ng/mL) Mean±SD
NC	180.46±245.91[a]
PC	414.22±13.46[b]
P1	425.50±32.60[b]
P2	444.11±2.35[b]
P3	418,02±10.91[b]

Differences in superscript notation indicate a significant difference between groups at P<0.05

Qualitative analyses of damaged kidney cells were carried out by determining a score based on the percentage of damage, and the number of cell damage, according to Windahartono *et al.* (2013). Table 3 shows the histo-pathological observations of rat kidney.

Table 3: Histopathological findings observed in the kidney tissues of the rats according to the experimental groups

Group	Necrosis	Inflammatory cells	Haemorrhage
NC (A)	-	++	-
PC (B)	+++	+++	+++
P1 (C)	+++	++	++
P2 (D)	+++	++	++
P3 (E)	++	+++	+++

(-) none, (+)1%-2.99% ,(++)3%-4.99%, (+++)5%-6.99%

According to Khairan (2010), biochemical processes in the body, such as inhalation of oxygen, aerobic metabolic processes, and excessive food processing will produce low amounts of free radicals, one of the products of free radicals produced, called malondildehyde. MDA

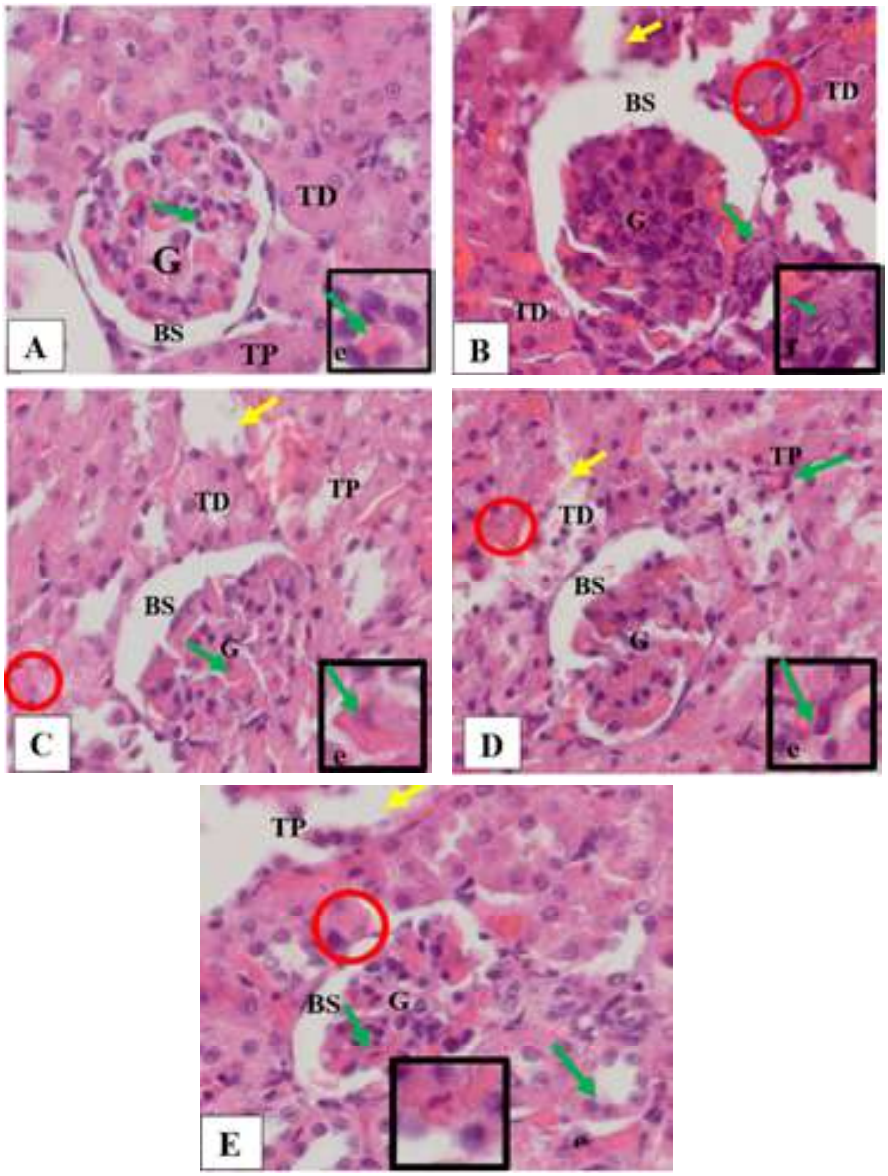


Fig. 1: Rat's kidney histopatological appearance. Group NC (A), Group PC (B), Group P1 (C), Group P2 (D), Group P3 (E): HE staining; Magnification 400x and 1000x (e).G (glomerulus); BS (bowman's space); TD (distal tubule); TP (Proximal tubule); (green arrow) = erythrocytes; (yellow arrow) = necrotic cells, e : Neutrophils, f : lymphocytes; red circle = haemorhage.

levels in the PC group exposed to TCDD at a dose of 200 ng/ kg b.wt., showed a significant increase ($P<0.05$) compared with the NC group. This proves that TCDD exposure has an effect on the kidneys, which can cause oxidative stress. TCDD has lipophilic properties; when it enters the body, it easily accumulates in the adipose tissue, making it difficult to remove. The elimination time of TCDD in mice lasts 12-31 days and in humans lasts for years, so that with these properties, TCDD will continue to be in the kidney adipose tissue for a long time and will continue to initiate oxidative stress (Rozewicz, 2016).

Based on the average MDA level, there was no preventive effect of fortified yogurt with purple roselle extract on the damage caused by dioxin intoxication in rat's kidney.These results can be caused by several reasons, including the concentration factor of purple roselle extract fortification, which at the given concentration did not have an effect on preventing the increase in MDA levels of the kidney due to dioxin intoxication. The analyses on the antioxidant activity test of yogurt fortified a concentration of 0.5%, 1.0%, and 1.5% purple roselle extract had a relatively low value of antioxidant levels (Table 1). Based on the antioxidant activity value of IC_{50} according to Alfian and Susanti (2012), the IC_{50} value is said to be very strong if it is less than 0.01 mg/mL, strong 0.01-0.05 mg/mL, moderate at 0.05-0.1 mg/mL, and weak if the value is more than 0.01 mg/ mL. For this reason, a higher concentration of purple roselle extract is needed in order to have an efficient effect on preventing increased levels of MDA in rat kidneys.

Pathological changes in the kidney tissues of the positive control group (PC) given with TCDD at a dose of 200 ng/kg b.wt., had a visible difference compared with the negative control (NC). The PC group showed necrosis that was present in almost all tubules, there are several inflammatory cells (neutrophils and lymphocytes), the size of the glomerulus is reduced, and the bowman space looks very wide. Reyner *et al*. (2016) explained that the blood flow to the kidneys is very large, and the kidneys also function in the excretion of metabolic products and foreign chemicals, so it is very risky to carry toxic materials and will cause damage to kidney tissue, especially in structural changes, kidney structure, and function.

Glomerular atrophy caused by TCDD deposited in the adipose tissue will indirectly cause the kidney to experience a gradual injury over a long period of time, then haemodynamic changes will occur and will trigger atrophy in the glomerulus, the space between the glomerulus and bowman's capsule will be filled with a lot of sediment causing the glomerulus is compressed and shrinks so that Bowman's space is widened. In the kidneys, haemorrhage is also found. Haemorrhage occurs due to TCDD, which is a toxic substance that damages blood vessels, so that blood and blood components go out into the tissue, characterized by finding red spots on the tissue. When there is hemorrhage in the tissue, the cells do not get blood intake. The cells will experience swelling, after that there is denaturation of proteins by the cells, and the cells experience death, characterized by loss of cell nuclei and chromatin image, the nucleus looks denser, becomes crooked, more color dark (pycnosis), divided into torn fragments (karyorrhexis), and pale (karyolysis). This event will increase the load when there is the filtration of the glomerulus, and inflammation occurs. The inflammation that occurs will trigger the release of inflammatory cells. According to Ulilalbab *et al*. (2018), giving antioxidants, especially orally for 36 days, can play a role in neutralizing or protecting the effects of compounds due to free radicals. Meanwhile, regenerating the renal epithelium, especially the tubules, needs approximately 1 month or 28 days to repair themselves (Yokote *et al*., 2012).

Recent study concludes that, based on the MDA level and the histopathological features of kidney tissues in rats TCDD exposure can increase the MDA level and histopathological changes in all groups exposed to TCDD. The prevention using yogurt fortified with purple roselle extract concentrates at 0.5%, 1.0%, and 1.5% has no effect on preventing kidney damage due to dioxin intoxication. These results suggest that an increase in concentration on purple roselle extract might increase the antioxidant properties and the protective effect.

Acknowledgments

The authors acknowledge financial support from the Research Institution of Brawijaya University (LPPM UB) contract number 759.6/UN10.C10/2020

References

Alfian R and Susant H (2012) Determination of total phenolic content of methanol extract of Red Rosella flower petals (*Hibiscus sabdariffa* Linn) With Spectrophotometric Growing Place Variations. *Pharmacy.* **2**(1).

Ariviani S, Fauza G and Pawestri C (2018) Development of purple Rosella (*Hibiscus sabdariffa*) as an isotonic drink with antioxidant potential and able to increase body fitness. *Agritech*. **37**(4): 386.

Chen Linlin, Deng, Huidan, Cui, Hengmin Fang and Jing (2017) Inflammatodry responses and inflammation-associated diseases in organs.

Fiedler H (2003) Dioxins and Furans (PCDD/PCDF) *Environ. Chem*. **3**(26): 11-13.

Hau, Jann and Schapiro and J Steven (2014) Handbook of Laboratory Animal Science, Volume III : Animal Models. CRC Press. Chapter 2.

Indraningsihand Sani Y (2014) Detection of Trichloro dibenzo-p-dioxins and Trichloro dibenzofurans in Beef with Gas Chromatography Tandem Mass Spectrometry. *J. Anim. Vet. Sci.* **19**(4): 302-314.

Mahdi C, Hariyanto S and Pandaga MC (2017) Effect of goat milk yogurt for prevention hypercholesterolamic in animal model rat (*Rattus norvegius*) based on the expression of inducible nitric oxide synthase (INOS) and a description of liver Histopathology. *Indonesian J. Cancer Chemoprevention*. **7**(2): 38.

Noviatri A, Setianingrum A and Haskito AEP (2020) Organoleptic properties evaluation of purple *Hibiscus sabdariffa* L (Roselle) calyx extract-fortified yogurt. *J. Physics: Conf. Series*. **1430**(1): 8-13.

Nugroho P, Dwiloka B and Rizqiati H (2018) Yield, pH Value, Texture, and Antioxidant Activity of Fresh Cheese with Acidifying Ingredients Purple Rosella Flower Extract (*Hibiscus sabdariffa* L.). **2**(1): 33-39.

Rozewicz M, Bombik E, Janocha A, Lagowsaka K, Bednarczyk M (2016) Dioxin- their influence on human health and the contamination of products of animal origin.http:// www.elizbieta.bomik.uph.edu.pl. [10 September 2019].

Sakin F, Bulmus FG, Servi K and Popa L (2011) Protective effect of lycopene on oxidative stress induced by different doses of 2,3,7,8-Tetrechlorodibenzo-p-dioxin in brain, liver, kidney, and heart tissue of rats. *Farmacia*. **59:** 462-470.

Suharto ELS, Arief II andTaufik E (2016) Quality and antioxidant activity of yogurt supplemented with roselle during cold storage.

Media Peternakan. **39**(2): 82-89.

Susanti R (2004) Cellular Immune Response Against 2,3,7,8 Tetrachlorodibenzo-p-Dioxin Intoxication in Rats. *J. Indonesia Trop. Anim. Agri.* **29**(1): 21-26.

Ulilalbab A, Wiratmadi B and Adriani M (2018) Purple Rosella (*Hibiscus sabdariffa* Linn.) petal extract prevents hepatocyte degeneration in Wistar rats exposed to cigarette smoke. *Folia Medica Indonesiana.* **54**(2): 96.

Widyaningsih W, Sativa R and Primardiana I (2015) Antioxidant effect of ethanol extract of Green Algae (Ulva Lactuca L.) against malondialdehyde (MDA) levels and CCL_4-induced superoxide dismutase (SOD) enzyme activity. **12**(2): 163-175.

Windhartono, Kamal Z and Sasmito E (2013) Effect of Carrot (*Daucus carota* L.) infusion on uranium induced male rat kidney histopathology. *Yarsi Medical J.* **21**(1): 33-40.

Yokote S, Yamanaka S and Yokoo T (2012) De Novo kidney regeneration with stem cells. *J. Biomed. and Biotechnol.*

Hydroponics Reservoir Temperature Monitoring and Controlling System under Greenhouse Condition

Amy Lizbeth J. Rico

Abstract: ***An automated reservoir temperature monitoring and controlling system for hydroponic system was developed, calibrated and validated in this study. The automated monitoring and controlling system was developed to monitor and control the reservoir temperature of nutrient solution in hydroponic system. The greenhouse available at the Center for Hydroponics and Aquaponics Technology (CHAT) and locally available materials and hardware for the hydroponics and automation were used in the development of the system. These devices were designed and assembled based on the conceptual framework of the study. The reservoir temperature sensor sends signal to the microcontroller which triggers the turning on/off of water chiller and the mixer. The instruments used were calibrated prior to the performance evaluation and obtained calibration equation for the water temperature sensor is y = x + 0.37. Validation of the automated reservoir temperature monitoring and controlling system was done and the recorded maximum temperature is 31 °C and the minimum temperature is 24 °C. The lettuce planted during the validation has an average height of 14.61 cm and the average leaf count of 12 for the lettuce crops during the 4th week after planting. A total of 4.78 kg of lettuce crop was harvested with an average of 20.6 grams per lettuce crop was obtained. Based on the performance evaluation and validation done on the automated reservoir temperature monitoring and controlling system, it was found to be reliable. This system becomes useful in reducing labor cost, and allows for real-time monitoring of reservoir temperature, therefore increasing farmers' crop productivity and income.***

Index Terms: automation, greenhouse, hydroponics, reservoir temperature, sensor

I. INTRODUCTION

In the present scenario, almost everything can be controlled and operated automatically, but there are still a few important sectors in our country where automation has not been adopted or not been put to a full-fledged use, perhaps because of several reasons such as cost. Agriculture has been one of the primary occupations of man since early civilizations and even today manual interventions in farming are inevitable. Without automation in hydroponics, many growers spend approximately 15-30 minutes a day testing and correcting the system levels. This means that beginning growers will often spend more time on testing parameters until the farmers familiarize themselves with the nutrient levels needed. Also, farmers tend to over-correct one or two of the variables. The automated reservoir temperature monitoring and controlling system keeps the system levels stable and provides the optimal environment for the plants which results to bigger and healthier plants.

Hence, this study is conceptualized to develop an automated system by monitoring the reservoir temperature of the nutrient solution in a hydroponic system for optimum plant growth as this factor can greatly affect the growth of lettuce. Specifically, the study aimed to; (1) install an automated reservoir temperature monitoring and controlling mechanism for the nutrient solution, (2) evaluate the performance of the automated monitoring and controlling device, and (3) determine the response of lettuce on the automated monitoring and controlling device

II. MATERIAL AND METHODS

A. Conceptualization of the Study

The conceptual paradigm of the study is presented in Figure 1. The study aimed to monitor and control the reservoir temperature of the nutrient solution using hydroponic system under greenhouse condition. Through this process, time and labor can be saved as well as real time monitoring of the parameters can be achieved.

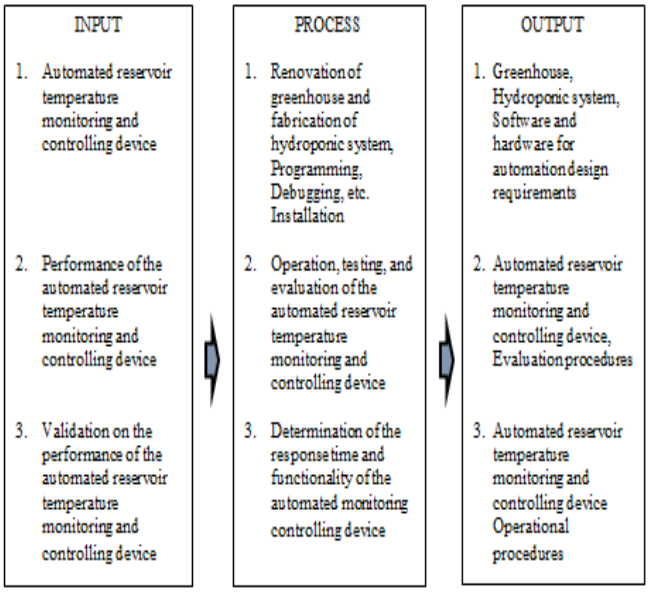


Figure 1. Conceptual framework of the study.

Revised Manuscript Received on September 25, 2019.
Amy Lizbeth J. Rico, College of Engineering and Technology, Tarlac Agricultural University, Malacampa, Camiling, Tarlac, Philippines 2306

B. The Production System

The automated hydroponic system used in the study is composed of the structural system, the hydroponic system, and the automation system.The automated reservoir temperature monitoring and controlling was tested in one of the greenhouse facilities located at the Center for Hydroponics and Aquaponics Technology (CHAT) measuring 3.0 meters in width, 4.0 meters in length, and 3.5 in meters height. The frames of the greenhouse are made from 2.54 cm galvanized iron pipes bended and welded together to form a Quonset-type structure. The structure is provided with three roof covers: the insect-proof net in the inner side, the ultraviolet- resistant plastic film in the middle and the gravy woven net shade on the outer side that offers strength and improve aerodynamics to withstand strong wind gust and heavy rains. The available water supply and power supply was used in the operation of the hydroponics system.

The recirculating tube culture system was used in hydroponic system. The hydroponic system was enclosed in the structural system. The grow pipes used was 300.0 cm in length and 0.075 cm diameter. A slope of 1 cm/100 cm of the pipe length was employed for the water to flow through the pipe with ease. The PVC pipes were drilled with 5.08 cm diameter holes and were spaced at 16.5 cm between holes (center to center) and made in 2-layer and 4-column pipe layout. A 150 L reservoir served as the source of water in the hydroponics system where the water was pumped to each growing tubes. The water flow in the hydroponic system was run by a 65-watt submersible pump, 1-2 liters/min flow for each growing tube that lifts the water to the upper layer of the growing tubes. A mixer inside the reservoir was installed to equally dispense the nutrient solution to the reservoir water.

Figure 2 shows the set-up of the automated pH monitoring and controlling device. The automation system served as the main component of the study and was composed of the controls, sensors, and hardware.

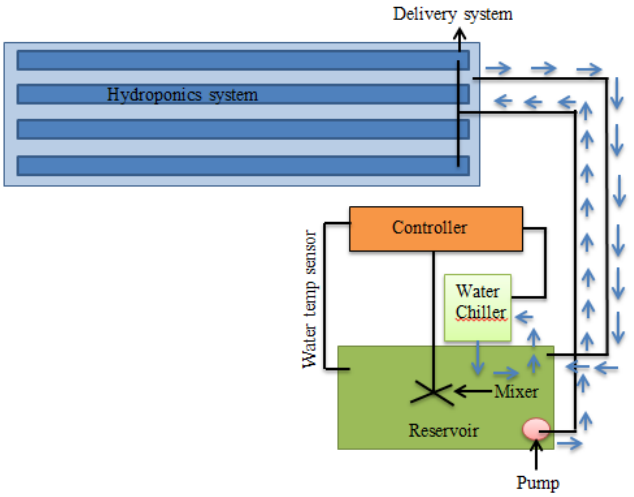


Figure 2. Set-up of the automated reservoir temperature monitoring and controlling device

C. Automation of the Reservoir Temperature Monitoring and Controlling Device

The automated reservoir temperature monitoring and controlling device basically monitor and control the temperature of the nutrient solution in a hydroponic system under greenhouse condition. Sensors were used to determine the reservoir temperature in the reservoir. The block diagram shown in Figure 3 is the layout of the hardware design that was used for the automated monitoring and controlling device. A microcontroller using the Arduino platform was used in programming the automation of the reservoir temperature monitoring and controlling device. Using this data, the microcontroller adjusts the temperature of the water in the system by turning on the mixer and the water chiller

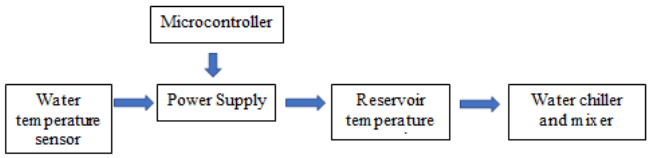


Figure 3. Block diagram of the automated reservoir temperature monitoring and controlling system

Shown in Figure 4 is the flow diagram of the automation used in the study. The LCD is initialized when the automation system is turned on. The reservoir temperature range of 24ºC - 30ºC for the nutrient solution was entered in the system. These ranges determine when the chiller and the mixer will be turned on. and determined using the water temperature sensor submersed into the reservoir. If the reservoir temperature reading is above 30ºC , the sensor sends signal to the microcontroller to trigger the chiller and the mixer to turn on. When the entered reservoir temperature range is attained, the sensors send signal the microcontroller to turn off the chiller and the mixer.

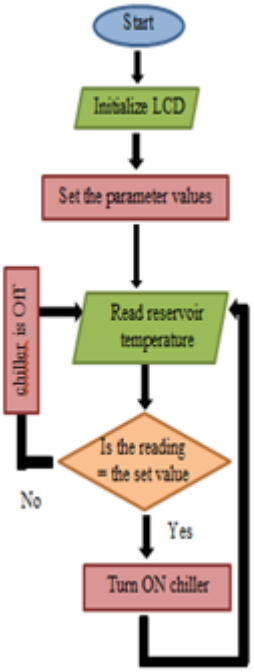


Figure 4. Flow diagram of the automated system

D. Calibration of the Water Temperature Sensor

The water temperature sensor was calibrated in order to achieve precision and accuracy.

The hourly reading for 24-hour period in the sensor was compared with the reading from the calibrated instruments. The difference in reading from the sensor and the calibrated instrument were recorded and graphed. Linear regression of the sensor reading and the calibrated instruments was obtained. The equation from the linear regression was inputted into the program for the water temperature sensor.

E. Final Testing

The reservoir temperature was monitored every day based on their response to the whole system. Automatic turning on of the device when the parameters are beyond the threshold range, response time of the device to be able to attain the threshold range, and automatic turning off of the devices when threshold range is attained were among the data gathered and recorded.

F. Lettuce Production

The leafy variety of lettuce (*Lollo rossa*) was used as planting material in the automated hydroponics system as this is commonly used as planting material in hydroponics system. Media composed of carbonized rice hull, sand and rice hull was used as planting media in the automated hydroponics system since these contain most nutrients needed by the plants. The planting cups containing 2-3 lettuce seeds were placed in cups. The cups were placed on individual cut-outs of the growing tubes. The net cups should touch the flowing water in the growing tubes to avoid the plants to be dehydrated. The pump continuously lifts the water and nutrient solution allowing the roots to avail of the nutrients. The reservoir temperature level of the nutrient solution was maintained at a range of 24ºC - 30ºC level which is the recommended reservoir temperature level for lettuce production under hydroponics system. At this reservoir temperature level, the needed nutrients were made available to the lettuce plants. These parameters were maintained throughout the growing stage until harvesting stage of the lettuce. The lettuce was harvested 27 days after planting.

G. Validation

Validation refers to the process of checking that a system meets the specifications and that it fulfils its intended purpose. In the automated hydroponics system, the data gathered from the final testing was analysed and graphed. The automation system was modified to optimize the production system based on the data gathered. Another growing cycle of the lettuce was planted in the automated hydroponics system. Response of the system was monitored from planting to harvesting of the lettuce. The gathered data during validation was compared from the gathered data from the final testing. The differences from the two growing cycle and their relationship was obtained.

III. RESULTS AND DISCUSSION

The microcontroller used in the automated hydroponics system is Arduino Mega 2560 which served as the brain of the system and served as the trigger. It also processes the sensor data. Most of the parts were connected to the Arduino using simple jumper wires and the wires were soldered to ensure that they would not get loose. All of the electronic parts were then placed into plastic enclosure to protect delicate electronic parts from dust and moisture.

A. Installation of the Automated Reservoir Temperature Monitoring and Controlling System for Nutrient Solution

The microcontroller used in the automated hydroponics system is Arduino Mega 2560 which served as the brain of the system and served as the trigger. It also processes the sensor data. Most of the parts were connected to the Arduino using simple jumper wires and the wires were soldered to ensure that they would not get loose. All of the electronic parts were then placed into plastic enclosure (Figure 5) to protect delicate electronic parts from dust and moisture.

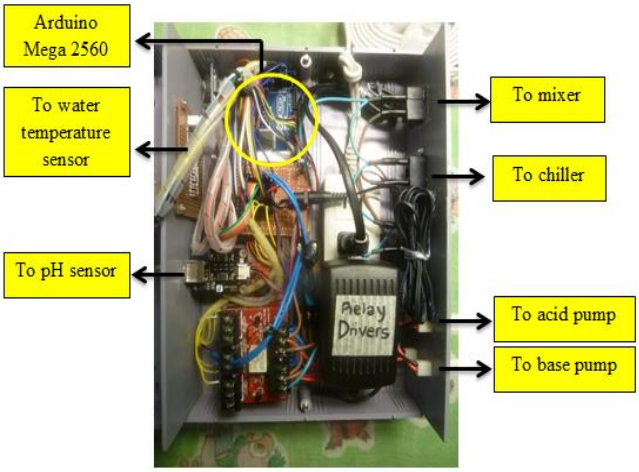


Figure 5. Electronic parts used in the automated hydroponic system

B. Water Temperature Sensor

The DS18B20 water temperature sensor shown in Figure 6 was used to determine the temperature of the reservoir in the hydroponics systems. The water temperature sensor was submerged to the reservoir and sends trigger signals to the microcontroller to activate the chiller thermostat and the mixer in the reservoir.

Figure 6. The water temperature sensor used in the study

C. Calibration of the pH Monitoring and Controlling System

Calibration of the reservoir temperature sensor used was done at the Center for Hydroponics and Aquaponics Technology in a 24-hour period before the data gathering. The reading from the sensor and calibrated instrument was obtained, recorded and graphed.

The graph of the calibration for the reservoir temperature sensor is shown in Figure 7. The graphs show linear relationship between the sensor reading and the instrument reading which also obtained an r^2 of 0.84. Based on the data gathered, the calibration equation for the reservoir temperature is $y = x + 0.37$. This equation was inputted in the program for the automation of the hydroponics system.

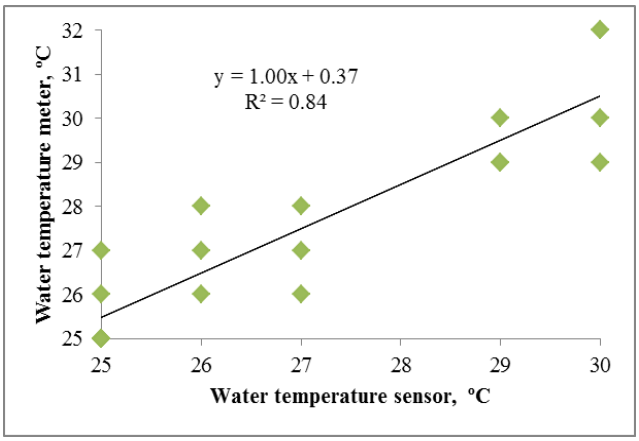


Figure 7. Calibration curve for water temperature sensor

D. Performance Evaluation

Based on the results, the obtained maximum reservoir temperature is 31ºC and minimum is 22ºC. The ability of the system to respond to the set threshold level, the response time of the system to the parameters, and the difference from the calibrated instrument were observed to be able to determine the reliability of the automated hydroponics system. Results showed that turning on of the chiller and mixer when the reading is beyond the threshold range is attained immediately after the reading is beyond the set value in the hydroponics system.

E. Validation of the Automated Reservoir Temperature Monitoring and Controlling System

During the validation period, the system was observed based on the criteria set for the reservoir temperature of the nutrient solution. Based on the results, the reservoir temperature reading and responses were accepted during the validation. Similar performance of the system during the validation and during the performance evaluation was observed. During the validation of the automated temperature monitoring and controlling system, the growth and number of leaves of the lettuce (test crop) were gathered and recorded weekly and the yield of the lettuce was obtained during harvesting. The lettuce crops obtained a total yield of 4.78 kg and an average of 20.6 grams per crop.

IV. CONCLUSIONS

Based on the objectives, the following conclusions were drawn:

1. the installed automated reservoir temperature controller was able to maintain the desired condition for the hydroponic system;
2. based on the observed successes and failures in monitoring the reservoir temperature, the performance of the developed automated reservoir temperature controller was found to be reliable, and;
3. the automated reservoir temperature controlling and monitoring device was able to grow lettuce with yield and responses similar to normal growing conditions.

REFERENCES

1. Anderson, M. (1989). Understanding Hydroponics. Volunteers in Technical Assistance, Inc. InternationL Journal of Applied Engineering Research. Volume 2.
2. Davis, E. M. & Kendall, A. D. (2014). The problem of the control system for Greenhouse Climate. Chinese Agricultural Science Bulletin. p154-157.
3. PAES. (2001). Greenhouses. Philippine Agricultural Engineering Standards Volume II. AMTEC, CEAT, UPLB, College, Laguna. Pp. D131 to D150.
4. Rico, A. L. J. (2019). Automated pH and Reservoir Temperature Monitoring and Controlling Systems for Hydroponics Under greenhouse Condition. Disseration. Central Luzon State University. Science City of Munoz, Nueva Ecija.
5. Karat, R. S. (1997). Application of the Wireless Sensor Networks in Agriculture, Transactions of the CSAE. p232-234.
6. Sace, C. F. (2013). Sustainable Agricultural Technologies. CERDS, CLSU, Munoz, Nueva Ecija.
7. Zabeltitz, E. C. (1997). Software Process and Product Improvement, A Historical Perspective, International Journal of Cybernetics, Volume 1, No1, Jan 2003 pp172-197.

AUTHORS PROFILE

The author was born on March 30, 1984 in Camiling, Tarlac. She is the second among the five children of Mr. Carlos O. Rico and the late Mrs. Estela J. Rico. She finished her elementary education at the Camiling West Central Elementary School in 1996 and took secondary level of education at the Tarlac College of Agriculture-Laboratory School, Camiling, Tarlac in 2000. She earned her Bachelor of Science in Agricultural Engineering degree from the University of the Philippines-Los Baños major in Land and Water Resources Engineering and Technology in 2005 and passed the Licensure Examination for Agricultural Engineer the same year. Months after, she was able to work as an Instructor at the then Tarlac College of Agriculture (now Tarlac Agricultural University). In 2009, she decided to pursue a higher degree and enrolled at the Central Luzon State University under the Master of Science in Agricultural Engineering, blessed to be accepted as one of the scholars in the Engineering Research, Development and Technology of the Department of Science and Technology (DOST-ERDT). Through hard work and dedication, she was able to finish her MS degree in 2014. With her goal to further enhance her skills and competencies, she pursued a PhD degree program in Agricultural Engineering at CLSU, again, through the Engineering Research and Development for Technology Scholarship Program of the Department of Science and Technology.