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

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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

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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.

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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

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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

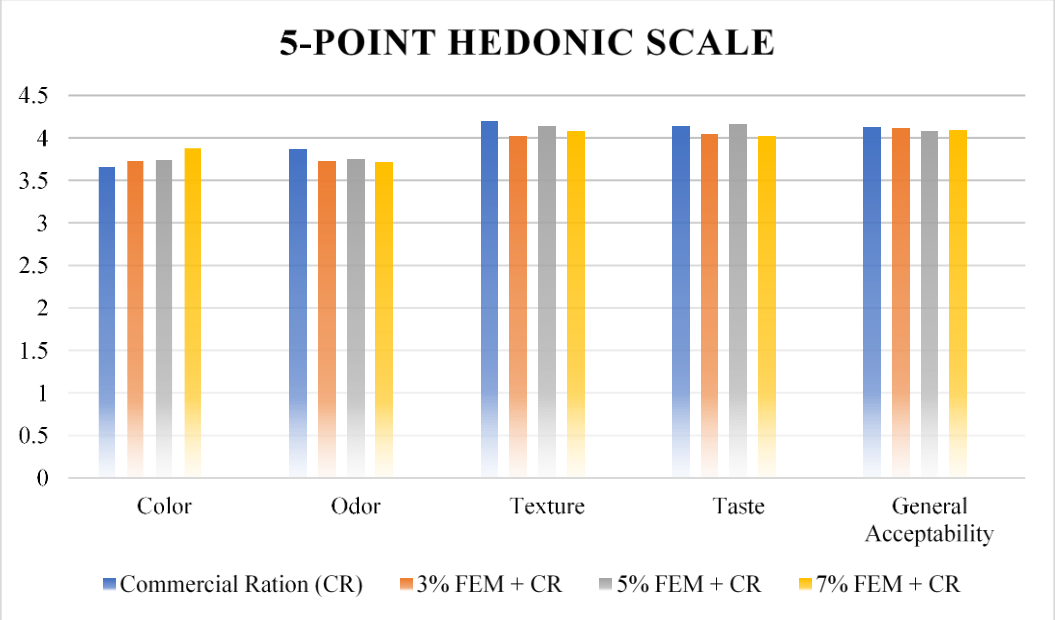

Figure 1. Organoleptic evaluation result of poultry meat as influenced with FEM by their ration based on 5-point scale score card.

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.

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

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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].

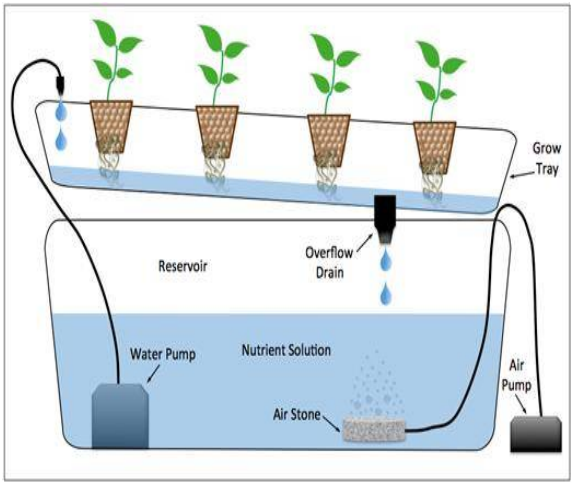

Figure 1. Diagram of the Nutrient Film Technique (NFT) hydroponic system [12]

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].

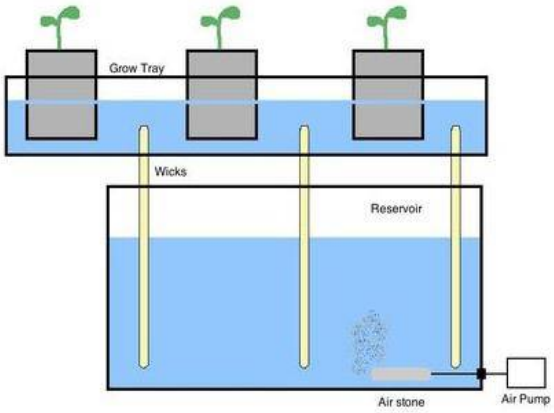

Figure 2. Diagram of the Wick System [14]

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].

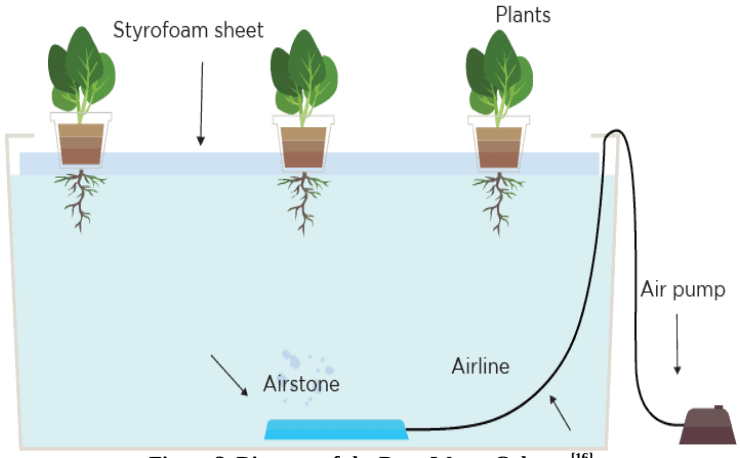

Figure 3. Diagram of the Deep Water Culture [16]

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].

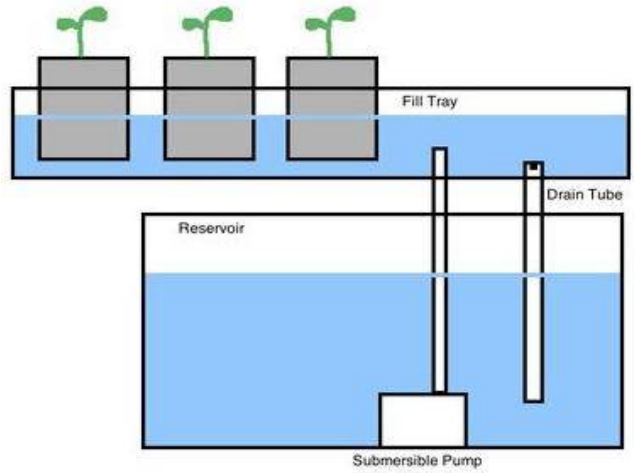

Figure 4. Diagram of the Ebb and Flow [18]

Aquaponics Farming

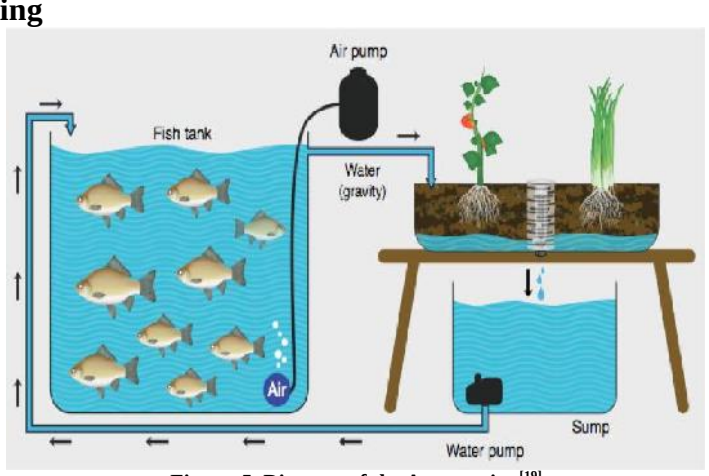

Figure. 5. Diagram of the Aquaponics [19]

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].

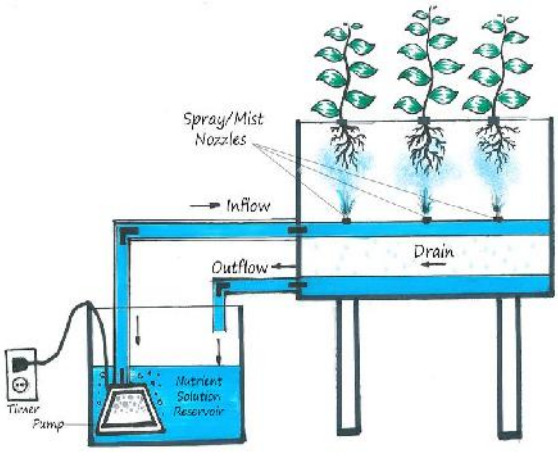

Figure 6. Diagram of Aeroponics [22]

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.

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FULL PAPER

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

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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).

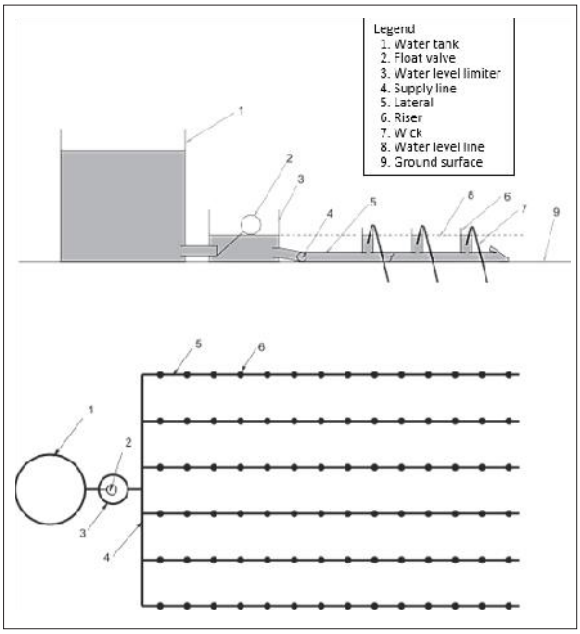

Figure 1. Schematic layout of the *capillarigation* system (Orge and Sawey, 2017)

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.

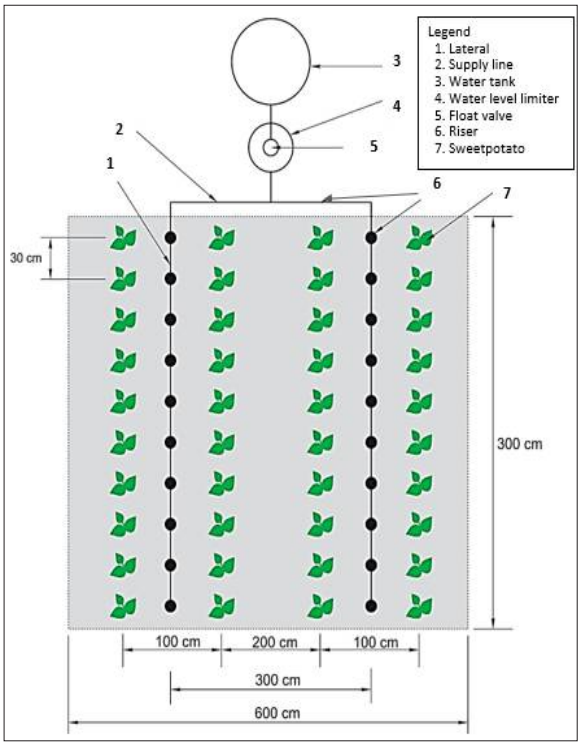

Figure 2. Layout of the *capillarigation* system established for sweetpotato production in the study.

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.

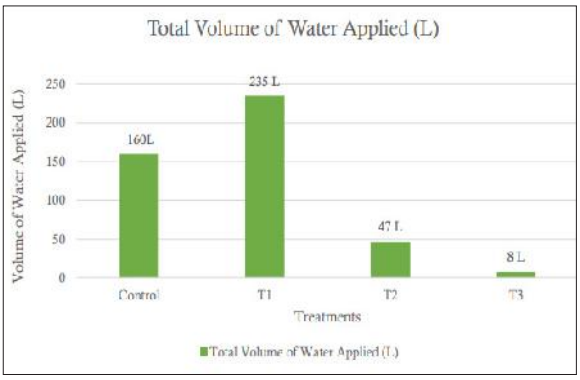

Figure 4. Total volume of water applied in each of the treatments.

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	*

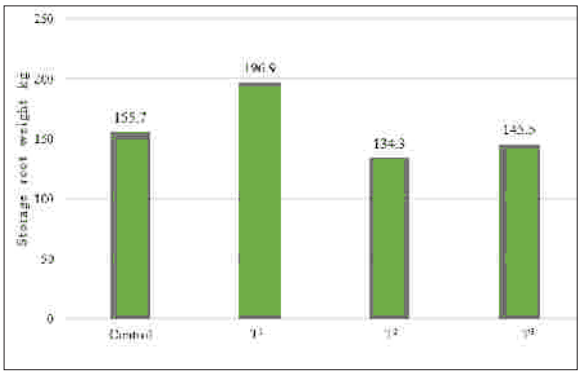

Figure 5. Total actual weight of harvested sweetpotato storage roots from each treatment.

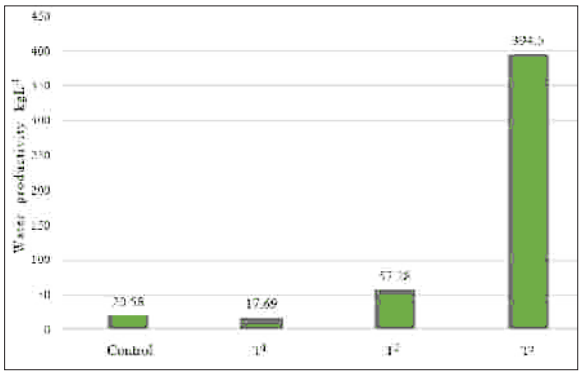

Figure 6. Water productivity of each treatment.

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.

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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

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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*.

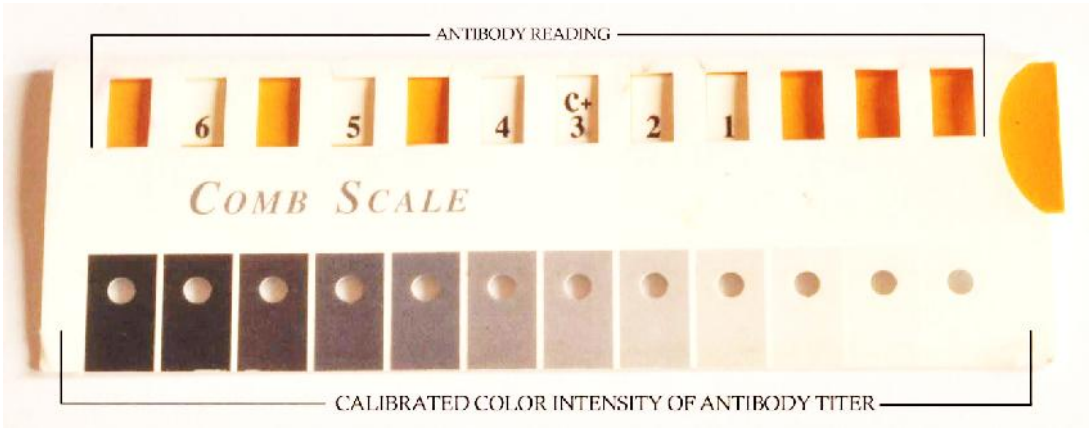

Figure 1. Comb card number 1, shows the purple-gray color results on each comb that corresponds to an antibody titer, the positive and negative control.

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

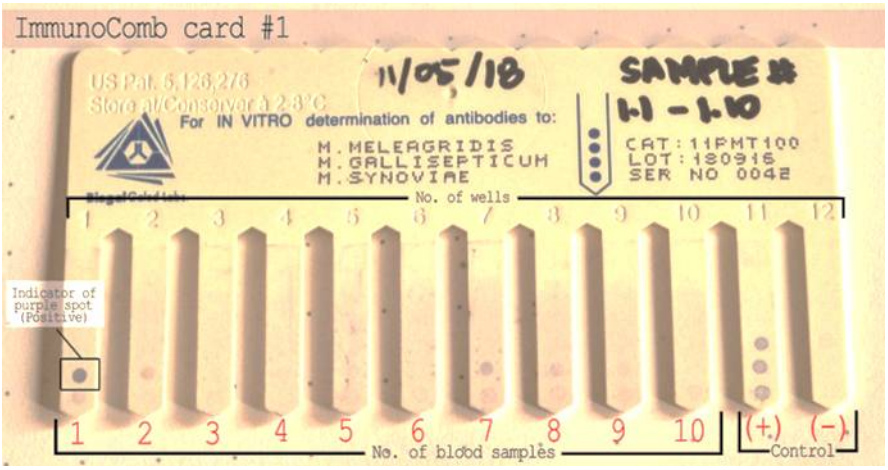

Figure 2. The Combscale shows the different color intensities that correspond to a particular antibody titer or level for each test.

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.

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

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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.

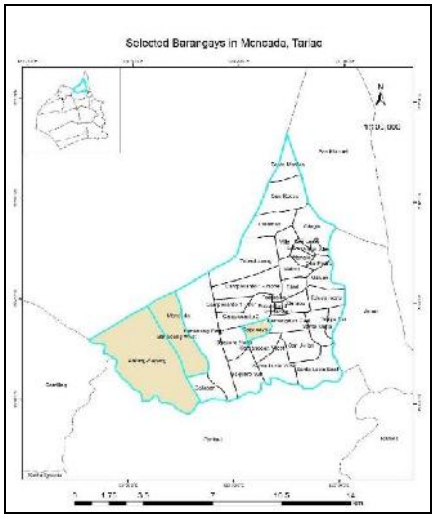


Fig. 1: Map of Moncada, Tarlac, Philippines.

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

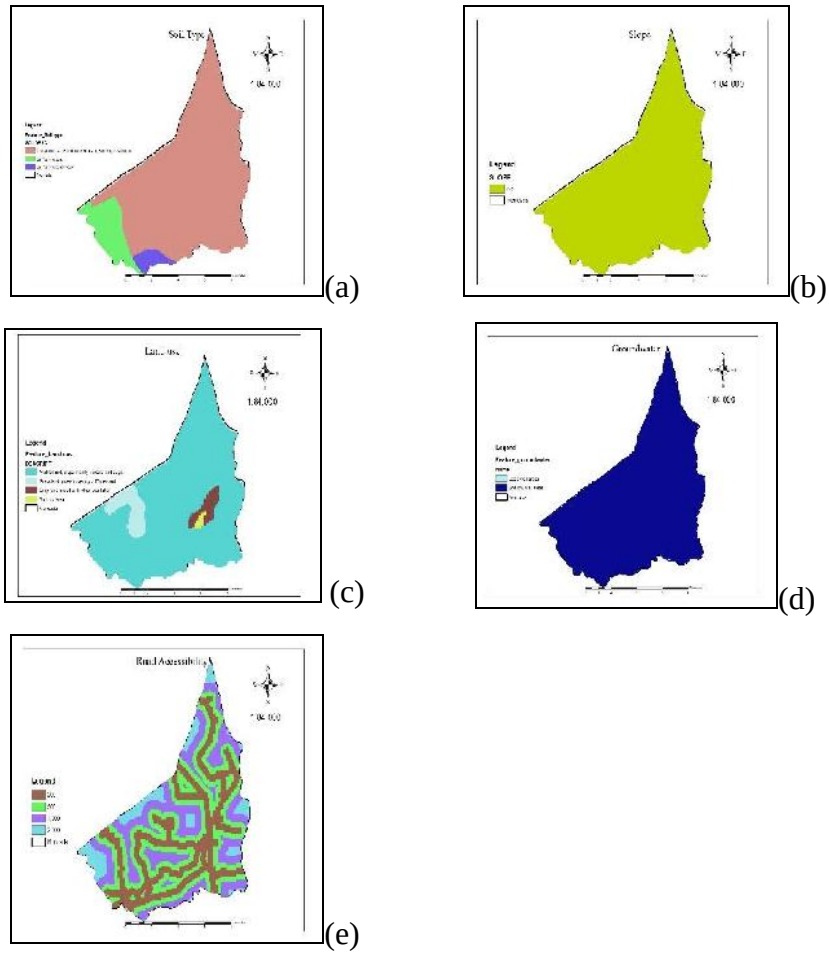


Fig. 2: Site Suitability Parameters Maps (a) Soil type Map (b) Slope Map (c) Land use Map (d) Groundwater Map (e) Road accessibility Map.

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.

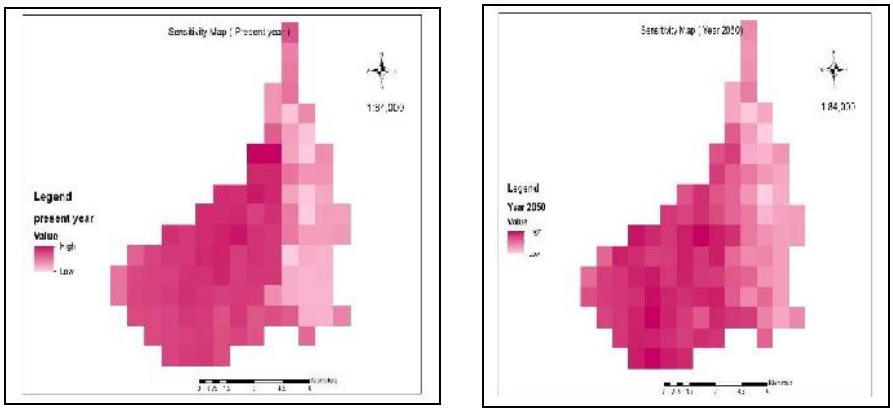
Class	Suitability Score
Highly Suitable	14-13.0001
Moderately Suitable	13-12.0001
Suitable	12-11.0001
Less Suitable	11-10.0001
Not Suitable	10-0.0000

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).

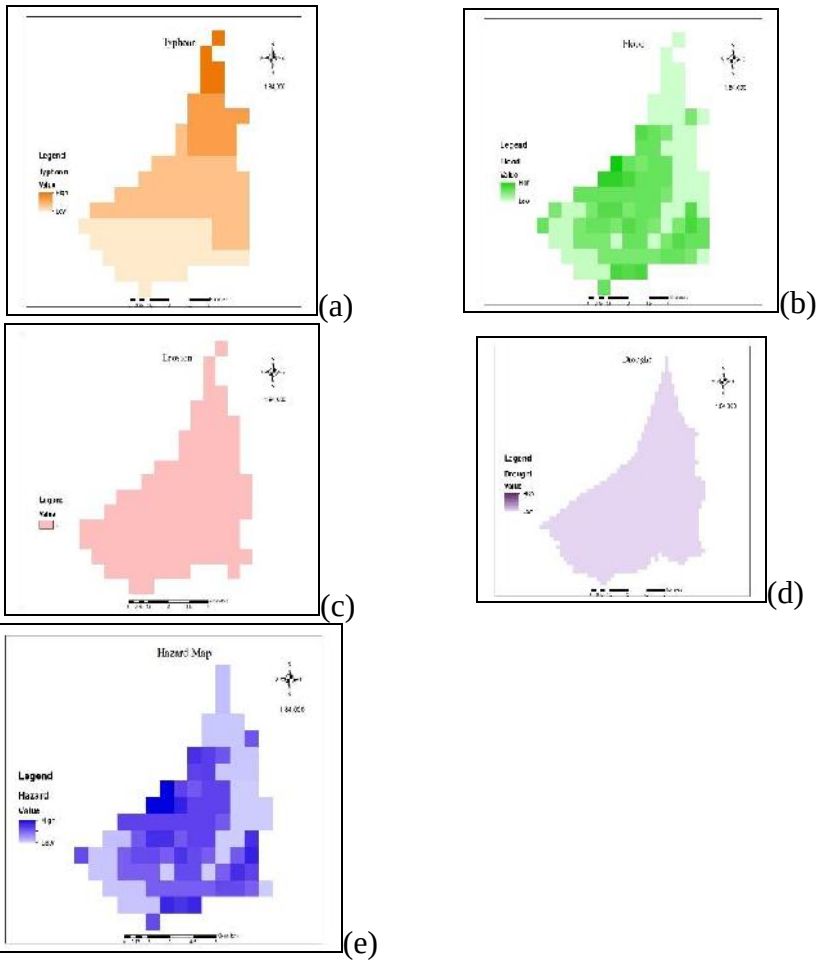


(a) **(b)**

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.



(a) (b) (c) (d) (e)

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).

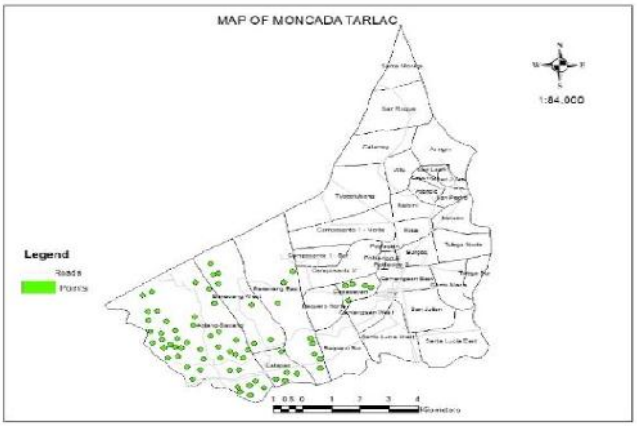


Fig. 5: Sweetpotato Production Areas with 3 Selected Barangays.

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).

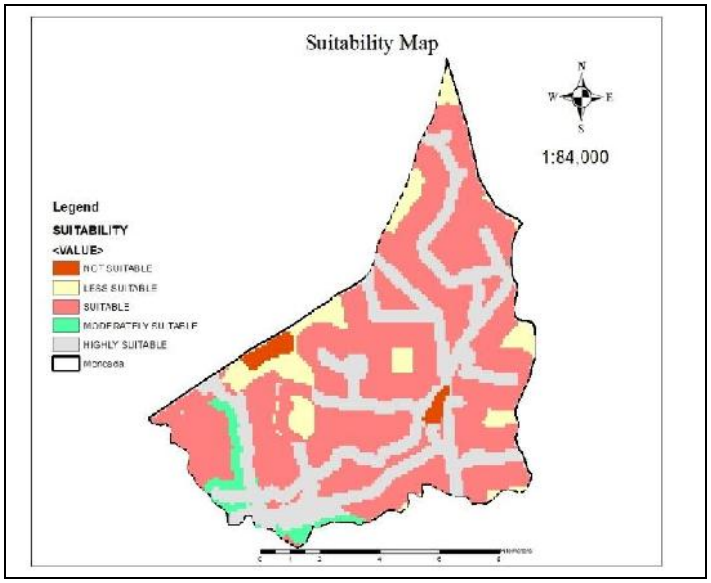
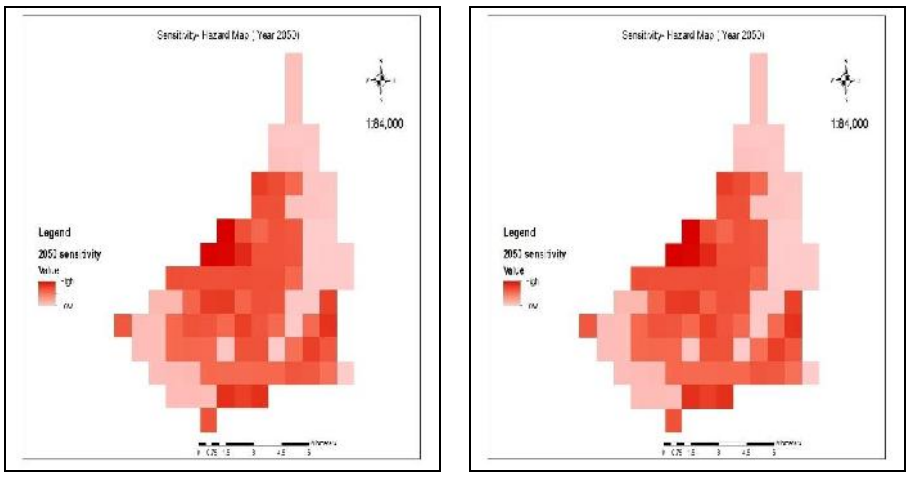


Fig. 6: Site Suitability Map of Sweetpotato in Moncada, Tarlac, Philippines.

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.

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AJAB

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Original Article

Molecular characterization of lactoferrin gene as genetic marker to subclinical mastitis in water buffaloes (*Bubalus bubalis*)

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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

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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

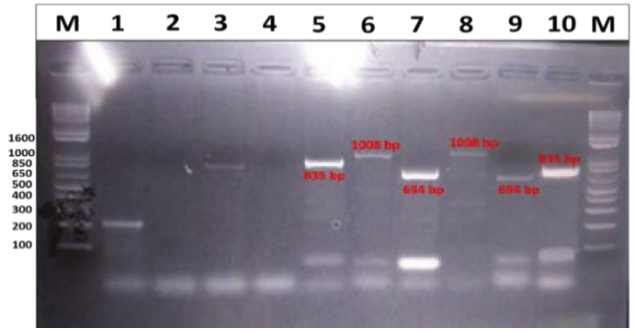

Figure-1: Amplification of Lf gene of swamp-type and riverine-type water buffaloes using primers 1, 2, and 3. Lane M (1kb plus ladder); Lanes 1-4 unamplified PCR products. Lane 5 and 10 (Primer 1); Lane 6 and 8 (Primer 2); Lane 7 and 9 (Primer 3)

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.

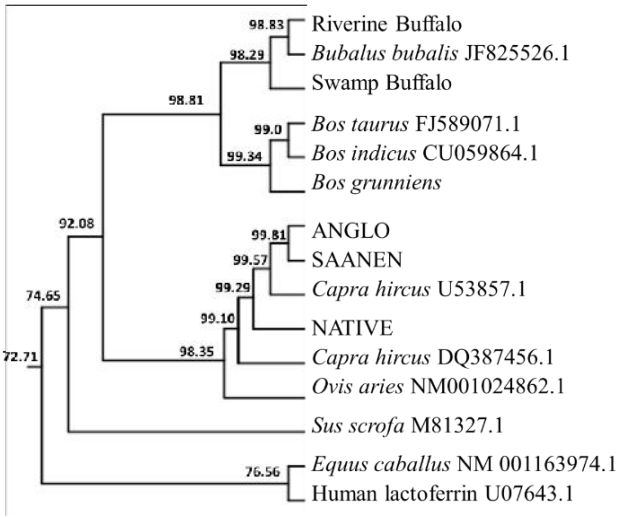

Figure-2: Phylogenetic tree showing the relationship of Lf gene between water buffaloes, goats, other ruminants' and human

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).

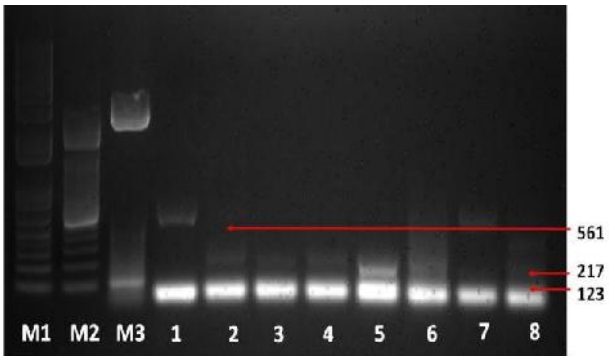

Figure-3: Restriction patterns of Bubaline Lf gene PCR product using *AluI* in 2% agarose gel. M1. 1000 bp ladder, M2. 100 bp ladder. M3. 25 bp ladder, Lanes 1 and 7 genotype AA (fragment size: 561, 123 bp), Lanes 2, 3, 4 and 5-genotype AB (217, 123 bp), Lanes 6 and 8-genotype BB (size: 561, 217, 123, 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.

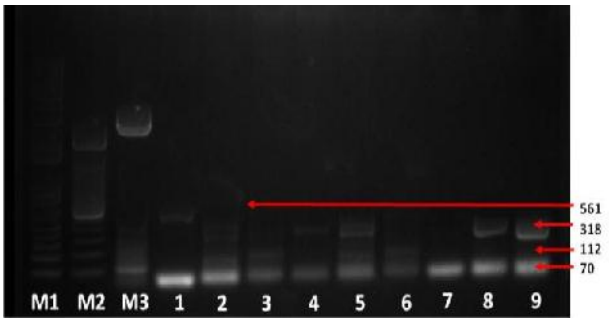

Figure-4: Restriction patterns of Bubaline Lf gene PCR products using *HaeIII* in 2% agarose gel. M1. 1000 bp + ladder, M2 100 bp ladder. M3 25 bp ladder, Lane 1 and 7, genotype AA (fragment size: 561, 70 bp), Lanes 2, 3, 5 and 6, genotype AB (fragment size: 561, 318, 112 and 70 bp), Lanes 4, 8 and 9, 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.

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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