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TECHNICAL EFFICIENCY, HOUSEHOLD INCOME, AND DEFORESTATION MITIGATION AMONG OIL PALM SMALLHOLDER IN SOUTH TAPANULI, INDONESIA

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ABSTRACT

Oil palm smallholders usually have low yield encouraging them to extend land areas. Some studies suggested increasing yields can lead to higher income and prevent smallholders from extending land areas. This paper aims to analyze the correlation between oil palm smallholders' Technical Efficiency (TE), income, household expenditure and deforestation mitigation potential. Research was conducted in July 2021, involving 160 smallholders, selected using the disproportionate stratified random sampling method. South Angkola and Batang Toru in South Tapanuli, Indonesia were selected as the research locations. Data was analyzed using the stochastic frontier analysis. The mean of TE level of smallholders (0.8) implies that the yield level of oil palm only reaches 80% of potential yield. Landholding size, farmers' education, and group involvement influence significantly TE level. At current production level, farmers get an income about IDR 49 million/year. An additional income of IDR 6.3 million/year is needed to cover household expenditure, which is equal to 0.2 ha. With 4,142 oil palm smallholding households in South Tapanuli, the total reduction in land needs will be around 838 ha. At optimal level of production (TE=1), the income could increase to IDR 64.5 million/year. It is higher than the household expenditure, thus no additional land is needed.

Key words: Good Agricultural Practices, household income, independent smallholders, stochastic frontier

INTRODUCTION

Indonesia is the largest palm oil-producing country, with a total plantation area of more than 16.3 million hectares (Kementerian Pertanian RI 2019). The significant increase in the global demand for crude palm oil has driven the large-scale development of plantations. In 2009, the Indonesian Minister of Agriculture planned to double the oil palm plantation area from 9.7 million ha to 18 million ha by utilizing 53% of the degraded areas and 47% of the most suitable land for oil palm, least suitable for food cultivation, and containing the lowest carbon stock (Koh and Ghazoul 2010). However, a decade later, in 2020, Purwanto et al. (2020) found that the spread of oil palm plantations to forest areas is around 15-20%, and Kehati (2020) estimated that around 36% of the total independent smallholding plantations area are operated illegally as they are located in forest areas. In other words, the increase in oil palm production was likely still significantly come from the land expansions.

Many argued that land expansion could be avoided by increasing the productivity of oil palm plantations. Intensification programs have been applied nationwide to inhibit deforestation (Tomich et al. 2001; Angelsen 2010; Garrett et al. 2018). The average Indonesian oil palm plantation only realizes around 60-70% of its potential productivity (Siahaan 2017). Among oil palm producers, smallholdings are considered the group with the highest potential improvement, as they show the lowest productivity. On average, oil palm smallholdings in Indonesia still faced land inefficiency (Sari et al. 2021; Dalheimer et al. 2022). In 2020, the average productivity of smallholdings was 2.56 tons CPO/ha/year (equal to 13.49 tons FFB/ha/year), while the state and private companies were 4.09 (equal to 21.51 ton FFB/ha/year) ton CPO/ha/year and 3.50 ton CPO/ha/year, respectively (equal to 18.43 ton FFB/ha/year) (Directorate General of Estates 2021). With low productivity, smallholders need more land to earn more income for covering their household expenses (Sari et al. 2021; Rhebergen et al. 2018).

The oil palm smallholders' technical efficiencies lead to low productivity. stochastic frontier analyses (SFA) and Data Envelopment Analyses (DEA) are two models that are widely used in estimating TE. SFA is a parametric model that accommodates the stochastic term, while DEA is a non-parametric one that uses a deterministic model. In SFA, both producers' inefficiencies and random elements are considered in the estimation, while DEA only includes the producers' inefficiencies (Sultana et al. 2023). The decision to choose the SFA or DEA models depends on the choice of input and output variables and the characteristics of the data analyzed. The SFA model is introduced to address the argument that not all the deviation in production should be only associated with pure TE. This is most important in agricultural products, in which some influencing production factors are unpredictable or random (Bai et al. 2007). Using generated data, it was argued that cross-sectional SFA holds no advantages over DEA (Ruggiero 2007). In contrast, using empirical data in both models, SFA fits better with the observed data estimation in potato production (Sultana et al. 2023). When the unpredictability reduces with the greenhouse microclimate, deterministic and stochastic models are feasible for modeling agriculture products (Yang et al. 2019).

Besides the unpredictable error, the inefficiency error from SFA is also influenced by the given existing technology of the sample data. TE results from various studies are affected by farm heterogeneity due to region-specific characteristics, thus, are not always comparable (Wang and Hockman 2012). Previous studies in several smallholders' oil palm centers found low TE, between 0.65 to 0.85 (Hasnah et al. 2004; Fariani et al. 2018; Varina et al. 2021; Ismiasih 2018; Latzko 2020). In contrast, an average TE of 0.95 was revealed in North Mamuju, West Sulawesi, which is not an oil palm center in Indonesia (Puruhito et al. 2019). Therefore, additional information about the technical potential of each location is also important.

Low TE stems from a lack of knowledge, financial support, and economies of scale. Many smallholders still use illegitimate seedlings, improper fertilizers, and only own 2 - 4 ha of land (Woittiez 2017; Folefack et al. 2019; Harsono et al. 2011; Soliman et al. 2016). On average, high-quality seedlings can reach 9 tons of CPO/ha/year compared to the current CPO productivity of only 3 tons of CPO/year (Baskett et al. 2008). In addition, the current fertilizer application methods and doses cause the productivity of smallholders to reach less than 50% of its potential (Woittiez 2017; Soliman et al. 2016). Most smallholdings were still facing increasing returns to scale. Oil palm smallholders with larger land sizes tend to be more efficient than the smaller ones (Dalheimer et al. 2022; Hernández 2020). If smallholders can correct their inconsistencies in agricultural practices, yield improvement can reduce land use of oil palm smallholdings. Field schools (FS) are among the alternatives that have been chosen and are still widely proposed to improve agricultural practices and productivity, including among the oil palm smallholders (van den Berg et al. 2020; Chalil et al. 2020; Pramudya et al. 2022). Previous studies estimated that a 2% yield improvement can reduce land use of oil palm smallholdings by 1 Mha. In total, the CPO supply can increase by 75%, or 15–20 MT/year, and reduce up to 4-6Mha or 17% to 58% of the total smallholding land (Folefack et al. 2019; Woittiez 2017; Van der Laan et al. 2017). Interestingly, intensification and high TE do not always lead to a reduction in new land use. Higher land efficiencies result in low production costs, which gives incentives (substitution effect) and purchasing power (income effect) for producers to obtain more land (Paul et al. 2019). Such conditions can be seen in the U-shape TE, in which both less and more efficient farms use more land for their agricultural activities, increasing deforestation (Marchand 2012).

While issues of oil palm smallholdings in the forest areas stemming from poor agricultural practices and low income still exist, studies that analyze the correlation between the smallholders' land needs and their household expenditure and the potential to increase production through TE improvement are very limited. This study was conducted in South Tapanuli District, North Sumatra, Indonesia. From 2000 to 2018, South Tapanuli recorded 45,000 hectares of deforestation, with the highest factor being plantation activities at 16,181 hectares. If there is no change in management (business as usual), estimations show that smallholders reach less than 50% of their potential, and by 2037, an additional 3,578 hectares of forest area will be converted to plantations (Pravitasari 2020). Findings from this study are expected to contribute to the literature on the TE of smallholders, household expenditure needs, and deforestation. In particular, the purposes of this paper are to (i) estimate the TE level of smallholdings, (ii) estimate factors that cause low levels of TE in smallholdings, and (iii) estimate the potential reduction in land needs with increased smallholding efficiency.

METHODOLOGY

This research was conducted in 2021 in the South Tapanuli District, one of the oil palm centers in north Sumatra, with a total area of 9,536 hectares involving 4,142 households. This district was selected as it had approximately 82% high conservation value and high carbon stock value in its total area. All the smallholding plantations in South Tapanuli were owned and managed by independent oil palm smallholders, developing their plantations with little to no assistance. Improper use of seeds and poor plantation maintenance negatively impact the average productivity of smallholdings in South Tapanuli, averaging only 80% compared to the average of North Sumatra (Chalil and Barus 2021). Two sub-districts, namely Batang Toru and South Angkola, were selected as they were classified as Special Cultivation Areas (Kawasan Budidaya Khusus), and the smallholders had received the GAP improvement FS Program. Batang Toru is located close to the city, with a dominant of S3 land suitability, while South

Angkola is close to the forest, with a mix of S2 and S3 land suitability (IOPRI 2009). Therefore, this study selected these two locations to observe their possible impact on productivity and deforestation.

The FS participants consist of 700 smallholders from four sub-districts. Sample smallholders were selected using a clustered sampling method based on their location and participation in the FS. The total samples involved in this study were 160 smallholders, consisting of 80 samples from each sub-district representing FS participants and non-participants. The sample size was determined by Yamane's formula with a margin of error of 10%, giving 40 samples for each sub-district (Al-Subaihi 2003). For comparison purposes, 40 additional non-participants were randomly selected from each sub-district.

The smallholdings' TE level was analyzed using the stochastic frontier production function. The method of measuring TE of a firm by estimating production function of firms at frontier production function was proposed by Farrel (1957). The stochastic frontier production function was then developed by Aigner, Lovell, and Schmidt (1977) and Meeusen and van den Broeck (1977) to capture the measurement error in production function. Battese and Coelli (1995) developed the stochastic frontiers and models for the TE effects that can estimate all the parameters involved simultaneously, particularly for panel data. The stochastic frontier production function was defined as:

$$Yi = f(x_{ik}; \beta_k) \exp (V_i - U_i) \tag{1}$$

where: Y_i is the productivity of oil palm in kg/ha/year; F (x; β) is a Cobb-Douglas production function of vector X (nitrogen in kg/ha/year, phosphor in kg/ha/year, potassium in kg/ha/year, harvesting labor in man hour, number of plants per hectare, crop age in year, and participation in the field school as dummy variable, 1 if involved in the field school, otherwise 0); Vector β (unknown parameter); V is random errors that were assumed to be independent and identically distributed (i.i.d.) N (0, σ_v^2); U is a non-negative random variable associated with the technical inefficiency of production, which was assumed i.i.d. U was obtained by truncation at zero from the normal distribution with mean, zδ, and variance σ_u^2.

The stochastic frontier model for this study uses cross-sectional data from all samples to estimate a single equation; it was assumed to be time-invariant. The parameters of the stochastic frontier and the model for TE effects were estimated simultaneously using the maximum likelihood method. TE has a value between 0 and 1, which was calculated using the following formula:

$$TE_i = \frac{Y_i}{Y_{i*}} = \frac{E(Y_i|U_i, X_i)}{E(Y_t|U_i = 0, X_i)} = E\left[\frac{exp(-U_i)}{s_i}\right] \tag{2}$$

where: Y_i is the actual productivity, and Y_i^* is the potential productivity.

The smallholdings' TE influencing factors were estimated using multiple regression, with TE as the dependent variable and eight variables as the vector of possible influencing factors. Technical inefficiency effects, U, is defined as:

$$U_i = z_i\delta + W_i \tag{3}$$

Z is a vector (1 x m) of free variables, namely Z_1 = land size (ha), Z_2 = education (year), Z_3 = smallholder experience (year), Z_4 = group involvement (1 if involved in smallholder groups, otherwise 0), Z_5 = smallholders' age (year), Z_6 = land status (1 if certified, otherwise 0), and Z_7 = family labor (%); δ is the vector (m x 1) of the unknown coefficients.

The potential reduction in land needs was analyzed by comparing the oil palm smallholding income in the current average TE with household expenditures. The income from oil palm plantations at the current TE was calculated using the average production and price. All prices, income, and costs were presented in IDR, which currency rate is 14,200 IDR/USD. Values from low, normal, and high seasons were multiplied by 0.3, 0.6, and 0.3, respectively. Household expenditures were calculated using routine and non-routine household expenses per year. The difference between income and household expenditures was then converted into the additional land needed based on the oil palm smallholding average income per ha. Then, the steps were repeated by calculating income with TE =1. The required land size was calculated by dividing the household expenditure/year by the oil palm plantation income/year/ha for the current and maximum TE.

RESULTS AND DISCUSSIONS

The TE of smallholdings. This study uses the Cobb Douglas production function. The specification was justified by the Ramsey Reset test result with F-stat 0.00, showing that linearity on the logarithmic data cannot be rejected at 1% significance. Before estimating the SFA with MLE, equation specification and assumptions on normality, homogeneity, autocorrelation, and heteroscedasticity for the production function were tested with OLS.

The residual scatterplot and PP plot showed a negative skewness, and the Kolmogorov-Smirnov test and Jarque-Bera test have 0.073 and 9.98 stat values at 1% significance. Therefore, the normality assumption is rejected. However, skewness residuals often appear in SFA, which have one-sided errors coming from inefficiencies. Commonly, production inefficiency errors have positive skewness, while costs have positive skewness. This condition means that the existing productivity is lower than the optimal frontier while the cost is higher. In this case, the negative skewness, -0.21, might partly be explained by the high difference in productivity between samples, stemming from the different land suitability and fertilizer usage. Some smallholders in South Angkola, which have S2 land suitability and use fertilizers very close to the recommended level, have very high productivity compared to those in Batang Toru with S3 and suitability and low-level fertilizer usage. Using the half distribution is argued to correct the inconsistent parameters due to the unnormal error distribution in OLS. This one-sided distribution includes half-normal, truncated, exponential, and gamma distributions (Carree 2002; Hafner 2016). In this case, the half-normal distribution was chosen for the production frontier estimation.

The Glejser and Breusch-Pagan-Godfrey homogeneity tests showed that all independent variables for the production function have coefficient regressions t-stat less than one, showing that they did not significantly relate to the residuals. However, the crop age has a t-stat of -2.27 and -2.29, thus rejecting the homogeneity assumption. Empirically, this can be explained by the oil palm crop productivity pattern that usually increases at age 3 to 10, then stays at the maximum for about ten years until 20, and starts declining until the end of its economic age of 25. The Durbin-Watson test gives a t-stat value of 1.68, with dL and dU values of 1.64 and 1.83, respectively. This result brings inconclusive results for autocorrelation. Empirically, there is autocorrelation of crop yield data due to spatial dependence of in-site specific crop management (Koutsos 2021).

The MLE production frontier estimation results showed that two of seven explanatory variables significantly influenced productivity, including the number of harvesting labor and the involvement of smallholders in the field school (Table 1).

Table 1. MLE results on the land productivity regression estimation.

Variable	Coefficient	t-statistic
Nitrogen (kg/ha/year)	-0.01	-0.79
Phosphorus (kg/ha/year)	0.00	0.27
Potassium (kg/ha/year)	0.00	-0.54
Harvesting labor (%)	0.09	2.49***
Number of plants/ha	0.02	0.20
Crop age (year)	0.04	0.94
FS Participant (dummy)	0.10	2.35***
Constant	2.69	5.01

Note: ** and *** = significant at 5% and 1%
Source: Primary data analysis

The empirical model of the stochastic production function is as follows:

$$y_i = 2.69x_1^{-0.01}x_2^{0.00}x_3^{0.00}x_4^{0.09}x_5^{0.02}x_6^{0.04}x_7^{0.10} \tag{4}$$

The involvement of smallholders in field school proved to be an essential factor in improving the skill and knowledge of smallholders to manage their farms, leading to an increase in oil palm production. Unfortunately, smallholders with larger land sizes are often reluctant to participate in the FS.

In contrast to several empirical findings in this case, fertilizer usage, plantation density, and crop age did not significantly affect oil palm productivity (Alwarritzi et al. 2015; Nordin et al. 2017; Ismiasih 2018). The gap between the current and the recommended fertilizer usage may partly be explained by the insignificant fertilizer coefficients. The potassium fertilizer, crucial for generative growth, had a gap of 33.50% and 66.61% below the recommended amount in South Angkola and Batang Toru, respectively. The fertilizer gap could also be negative, as shown in the average phosphate usage of participants in South Angkola. Overall, FS participants (FS P) show less fertilizer gap than the non-participants (NP) (Table 2).

Table 2. Average fertilizer usage and the gap to the recommendation.

Fertilizer	South Angkola				Batang Toru			
	Mean (kg/ha/year)		Gap (%)		Mean (kg/ha/year)		Gap (%)	
	FS P	NP	FS P	NP	FS P	NP	FS P	NP
Nitrogen	133.56	121.60	9.76	17.84	82.29	108.33	44.40	26.81
Phosphate	98.46	70.66	-9.29	21.57	69.58	65.55	22.77	27.24
Potassium	134.23	99.86	10.60	33.50	72.64	50.13	51.62	66.61

Source: Primary data analysis
Note: Recommended fertilizers refer to IOPRI (2022) for each type of macro fertilizer at the crop age range of 9-15 years

In some cases, the number of trees has the highest effect on production (Abdul et al. 2022). In this case, there was not much variation in the number of plants and age among smallholders. The number of plants in South Angkola is 116 and 122 per hectare, and in Batang Toru is 129 and 120 per hectare for participants and non-participants, respectively. Depending on the plant variety, the optimum plant density is around 130 to 143 (IOPRI 2022). Besides the quantity, the quality of seedlings should also be considered. Most smallholders do not use certified seedlings. In South Angkola, non-participants did not use certified seedlings, while only 7.5% of the participants used them. In Batang Toru, 37.50% of participants utilized certified seedings, while 15% of the non-participants do. The numbers show another opportunity to increase the smallholders' productivity by up to 300% by replacing illegitimate seedlings (Basket et al. 2008). The change can only be made during the replanting period. Therefore, a well-planned replanting program is crucial.

The estimation results need to be interpreted with caution as the R^2 productivity function is only 0.090, showing that most of the variation in the function is not explained by the independent variables. Such a condition was then further tested using a simple OLS regression estimation, which shows that more than 95% of the production level is explained by land size. The function also shows heterogeneity issues. These issues might be related to the high variation in fertilizer usage at the same age. Many of the samples have limited knowledge of the recommended fertilizer usage. In addition, the insignificant fertilizer usage has a negative phosphate gap, indicating their random usage. This gap shows the possibility to increase productivity by improving good agricultural practices. A similar low R^2 issue appears in several agricultural production and stochastic frontier functions studies. They are considered empirical rather than econometric issues. Therefore, the estimation results are still used to explain related causes (Abdulai 2018; Butzer 2011; Tauer 2006; Vollenweider 2016; Wu 2011). The TE estimation also showed that the coefficient of land size is significant at 1%. As this paper focuses on TE, productivity estimation is still used with empirical situations as the explanation.

Under such conditions, all smallholder samples achieved a TE value of more than 40%. Using the SFA, oil palm smallholdings that follow the recommended oil palm cultivation have a higher TE than those that do not (Ariyanto et al. 2020). Previous studies show that FS can improve farmers' technical efficiencies by promoting pro-adaptive behavior (Oguntade 2012; Saddozai et al. 2013; Purwasih et al. 2020; Zubair et al. 2021; Tomlinson and Rhiney 2018). However, the results do not strongly conclude the impact of FS on TE. In South Angkola, almost 95% of the non-participant samples have TE $\geq$ 0.80, and none have less than 0.60. The increase in knowledge through FS does not necessarily have a linear relationship with the increase in technology adoption (Huluka 2015). The knowledge can be translated into adoption only if enabling factors and conditions exist. In Batang Toru, FS participants have a slightly higher percentage of TE > 0.80 than non-participants (Table 3).

Table 3. Technical efficiency distribution frequency (percentage of the sample).

Technical efficiency level	South Angkola		Batang Toru	
Index	FS P	NP	FS P	NP
$0.40 \leq TE < 0.60$	2.56	0.00	2.70	5.00
$0.60 \leq TE < 0.80$	71.79	5.26	24.32	35.00
$TE \geq 0.80$	25.64	94.74	72.97	60.00

Source: Primary data analysis

This result contradicts the MLE estimation results of the land productivity function (Table 1), which shows that FS significantly influenced productivity. Therefore, the productivity of FS participants was tested before and after training. The productivity in South Angkola and Batang Toru increased by 30.07% and 36.70%, respectively. This improvement stemmed from the increase in their nitrogen, phosphate, and potassium usage by 58.11%, 67.50%, and 174.77% in South Angkola and 38.81%, 49.00%, and 52.68% in Batang Toru. However, data on FS participants before training were not used in TE estimation as they violate the requirement for independent decision-making units in SFA. Time-varying technical inefficiencies exist, and panel data are needed to capture technical changes (Battese and Coelli 1995; Dhawan and Gerdes 1997). Therefore, further work with panel data is required to obtain a robust conclusion.

The TE influencing factors. Estimation results revealed that land size, level of education, and group involvement significantly influenced the TE of the samples (Table 4).

Table 4. Factors affecting technical inefficiency.

Variable	Coefficient	t-statistic
Land size (ha)	-0.09	-3.31***
Education (year)	-0.02	-2.03**
Experience (year)	0.00	-0.48
Group involvement (dummy)	0.25	2.72***
Smallholder age (year)	0.00	-1.56
Land status (dummy)	-0.11	-0.99
Family labor (%)	0.07	0.81
Constant	0.63	2.51

Note: ** and *** = significant at 5% and 1%
Source: Primary data analysis

The inefficiency effects equation is as follows:

$$IE = 0.63 - 0.09z_1 - 0.02z_2 + 0.00z_3 + 0.25z_4 + 0.00z_5 - 0.11z_6 + 0.07z_7 \quad (5)$$

The negative coefficient of the land area and level of education means that a larger land size and a higher level of education led to less technical inefficiency. This result is in line with previous studies that found oil palm smallholders with larger land sizes tend to be more efficient than the smaller ones. The size and technical efficiency are partially explained by technological factors, such machines, fertilizers, pest control, irrigation systems, and technical assistance (Dalheimer et al. 2022; Hernández 2020). The positive coefficient of group involvement showed that group members had higher inefficiency than those who did not join smallholder groups. This result contrasts with many empirical studies suggesting the contribution of farmer group participation in increasing agricultural productivity and efficiency (Baga et al. 2023; Abdul-Rahaman et al. 2018; Agarwal 2018). In this case, many non-participants did not join any smallholder group. They showed less interest in joining groups or participating in the FS, as they have not seen significant benefits. Smallholders would actively participate in a smallholder group only if higher benefits were perceived (Ibnu et al. 2018). Family workers provide a positive impact due to better motivation and low management costs but a negative impact with their limited technical and managerial capabilities (Kostov et al. 2018). In this study, however, family workers do not significantly impact the TE.

TE and land size additional need. Given the TE, this study estimated the smallholders' additional land needs based on land productivity, household income, and expenditure. The average non-participant productivity in South Angkola is higher than FS participants and vice versa for Batang Toru. However, all groups did not reach their potential productivity level (Table 5).

Table 6 shows the difference in the average production and income of each group. The average income of smallholders is around IDR 34.10 million to IDR 80.50 million/HH/year. Except for non-participants in South Angkola, all groups have larger HH expenditures than their income. They needed an additional IDR 13 million to IDR 25 million/year/HH. To fulfill their needs, the smallholders need an additional IDR13.08 million to IDR21.17 million/HH/year. This is equal to an additional 0.56 to 1.11 ha of land per HH, or an average 0.20 ha/ HH.

Table 5. The gap between current and potential productivity.

Description	South Angkola		Batang Toru	
	FS Participant	Non-Participant	FS Participant	Non-Participant
Productivity (FFB tonnes/ha)	20.42	23.43	22.38	18.92
Crop age (year)	12.98	15.59	10.38	9.60
Potential productivity (FFB tonnes/ha)*	30.00	27.75	28.50	27.50
Difference in current and potential productivity (%)	32	15	21	31

* IOPRI, 2009
Source: Primary data analysis

Table 6. Current production, income and additional land need (TE = 0.8).

Description	South Angkola		Batang Toru	
	FS Participant	Non-Participant	FS Participant	Non-Participant
FFB production (ton/year/HH)	43.76	127.25	39.62	33.21
Selling price (IDR/kg)	1,287.06	1,156.16	1,457.87	1,483.44
Revenue (IDR/year/HH)	56,320,246.17	147,122,583.73	57,756,313.38	49,267,184.53
Costs of production (IDR/year/HH)	17,091,145.31	66,637,344.34	15,574,697.69	15,172,270.31
Income per HH (IDR/year)	39,229,100.86	80,485,239.39	42,181,615.69	34,094,914.22
Difference between FFB income and HH expenditure (IDR/year)	-16,036,327.71	25,219,810.82	-13,083,812.88	-21,170,514.35
Income per ha (IDR/year)	18,112,031.25	14,422,300.79	23,523,635.31	19,019,670.63
Additional land needed (ha/HH)	0.89	-1.75	0.56	1.11
Average additional land need (ha/HH)				0.2025

Source: Primary data analysis

If smallholders can overcome their constraints and reach the maximum TE (TE=1), they could significantly increase the HH income (Table 7). The income could reach IDR43.948 million/year/HH to IDR109.909 million/year/HH, thus their additional income needs reduce to IDR 1,533 million/year/HH to IDR -11,317 million/year/HH. On average their income is higher than their household expenditure, therefore they no longer need additional land. The increase in TE can reduce the potential expansion of smallholdings by around 0.20 hectares/household. With 4,142 smallholder households in South Tapanuli, the total reduction of deforestation risk in South Tapanuli is approximately 828 hectares.

The use of certified seeds can be another potency to increase productivity. The data showed that the use of certified seeds was still very low. In South Angkola, none of the non-participant samples use certified seeds, and only 7.5% of participants use certified seeds. Batang Toru showed better conditions, with 15% and 37.5% of the non-participants and participants using the certified seeds, respectively. Therefore, replanting should be prioritized to improve this condition. Certified seeds can increase productivity by 31.5% (Ardana et al. 2022). In this regard, Indonesia implemented the Communities' Oil Palm Replanting (Peremajaan Sawit Rakyat) program since 2018 to support smallholders in using certified planting material.

Table 7. Potential production, income, and additional land need (TE = 1).

Description	Angkola Selatan		Batang Toru	
	FS Participants	**Non-Participants**	**FS Participants**	**Non-Participants**
FFB production (ton/year/HH)	52.51	152.70	47.54	39.85
revenue (IDR/year/HH)	67,584,295.41	176,547,100.48	69,307,576.05	59,120,621.44
Income per HH (IDR/year)	50,493,150.09	109,909,756.14	53,732,878.36	43,948,351.13
Difference between FFB income and HH expenditure (IDR/year)	-4,772,278.48	54,644,327.57	-1,532,550.21	-11,317,077.45
Income per ha (IDR/year)	23,371,912.50	20,083,323.96	30,017,272.38	24,621,799.75
Additional land needed (ha/HH)	0.20	-2.72	0.05	0.46
Average additional land need (ha/HH)				0

Source: Primary data analysis

CONCLUSION

Smallholders' productivity is significantly influenced by harvesting labor and FS participation. The improvement in their input usage and TE leads to higher productivity and income. However, almost all smallholder respondents have not reached the optimal TE. Land size, education, and involvement in smallholder groups influenced significantly the TE of smallholders. The income of these smallholders is smaller than their household expenditures. Therefore, they need additional land to increase their income. If all the smallholders' agricultural practices can be corrected and reach maximum TE, they can earn more income than their household expenditures and no longer need additional land. This study demonstrated that FS has a positive impact in improving TE and could mitigate deforestation. However, the result is not strong enough to show the difference between the participants and non-participants. On the other hand, the data show a strong impact of FS participants' fertilizer usage and productivity before and after trainings. Further work with panel data is required to explain the indication of technical change among the FS participants.

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DIVERSITY OF BACTERIA IN PADDY FIELD SOIL AND THEIR CORRELATION WITH SOIL CHEMICAL PROPERTIES

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ABSTRACT

Soil bacteria play an important role in the decomposition of organic matter and nutrient cycling for rice growth. In tropical Thailand, the Ayutthaya soil series possessed unique characteristics after 8 years of continuous chemical fertilizer application, affecting different soil bacterial communities. Metagenomics analysis of bacterial diversity in rice fields and their correlation to soil properties were studied in Bang Phasi sub-district, Bang Len district, Nakhon Pathom province, Thailand (14.044116N and 100.237599E) in May 2022. Soil nutrient content and organic matter (OM) were measured. Bacterial taxonomy and a diversity index were defined. The correlations between bacterial genera and soil properties were calculated. Soil from the paddy field was very high in OM and quite high in available phosphorus. High bacterial diversity was found in the paddy field soil based on the Shannon index (8.30). The most common phyla were Proteobacteria (29.68%) and Epsilonaeota (21.67%). The genera *Sulfuricurvum* (21.58%) were predominant and correlated with the potential of hydrogen (0.95) and potassium (0.97) while *Thiobacillus* correlated with phosphorus (0.95). The genera *Anaerolineaceae*-uncultured associated with the total nitrogen (1.00) while *Bryobacter* positively linked with the OM (1.00). These bacteria relate to soil properties and can be isolated for biofertilizers in rice cultivation for possible reduction of the use of chemical fertilizers. Furthermore, this is the first study on the diversity and taxonomy of unculturable bacteria in the Ayutthaya soil series in Thailand using metagenomics analysis.

Key words:biodiversity, diversity index, metagenomics, rice field, soil bacteria,

INTRODUCTION

Rice is economically important and Thai people consume it as a staple food, with approximately 10 million ha cultivated in Thailand (Office of Agricultural Economics 2023). Paddy fields are mostly found in wide areas in the central region and along the river basins of Thailand, where there is often flooding during the rainy season (Soil Resources Survey and Research Division 2010). The sustainability of rice production systems globally is intricately related to the chemistry, physics and biology of rice soils (Prasanna et al. 2012). Various bacteria are important for rice growth. The most common group in paddy field soil was in the phylum Proteobacteria, followed by the phylum Firmicutes (Breidenbach and Conrad 2014). Bacterial communities in Brazil's flooded rice fields presented bacteria in the phyla Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi and Proteobacteria (Pittol et al. 2018). Several reports identified beneficial bacteria for rice production, including sulfur-oxidizing bacteria, iron-reducing bacteria, nitrogen-fixing bacteria, and nutrient-solubilizing bacteria (Kartik et al. 2023; Khan et al. 2017; Maeda 2021; Senthilkumar et al. 2021). In the paddy field, abundant sulfur-oxidizing bacteria were involved in oxidation at the soil surface and in the rhizosphere under different sulfur fertilizer conditions (Masuda et al. 2016). These sulfur-oxidizing bacteria increased the rice stem diameter, plant height and shoot weight (Pourbabaee et al. 2020) because sulfur plays an important role in plant metabolism, affecting photosynthesis, carbohydrate metabolism and pathways to protect against oxidative stress (Lunde et al. 2008). Iron-reducing bacteria produce ferrous compounds, which are regarded as crucial mediators of carbon and nitrogen processes in paddy soils and are affected by crop rotation, soil pH and the carbon -to- nitrogen ratio (Peng et al. 2016). Nitrogen-fixing bacteria fix nitrogen from the air and convert it into an ammonium form that rice can use (Doni et al. 2022). Bacteria oxidized elemental sulfur and reduced sulfur compounds to generate sulfuric acid that could solubilize insoluble phosphorous (Ullah et al. 2014). Phosphate-solubilizing bacteria promoted rice growth because they produced organic acids, enzymes, indoleacetic acid, siderophore and antagonists (Panhwar et al. 2012).

Several research papers reported soil management to improve soil bacterial communities such as, biochar addition to promote the nitrification process with higher community diversity (Hou et al. 2021), long-term input of pig manure modified soil abundance and rare bacterial and fungal composition (Hou et al. 2022), and green manures contributed to sustainable soil and nutrient management in agriculture and changed bacterial community structure (Songjuan et al. 2021). The relative abundances of functional genes responsible for nutrient cycles and the organic compounds degradation were enriched with cow manure, which have been continuously applied to paddy lands over 8 years (Wang et al. 2021a). Furthermore, land use change affects the physical and chemical properties of the soil, resulting in changes in the composition of the soil bacterial community (Sun et al. 2021). Soil organic carbon and total nitrogen contents, microbial biomass, and respiration intensity under different land uses were changed (Li et al. 2007). Jia et al. (2021) showed that bacterial diversity was positively associated with pH, soil nutrients and organic carbon. Soil carbon and nitrogen transformation and pH, cooperatively influence rice yield by regulating soil fertility (Wang et al. 2021b). Long-term use of fertilizers influenced structures of bacterial communities along a soil depth gradient in paddy soil and found *Nitrososphaera*, *Nitrospira*, and several members of *Acidobacteria* in topsoil in Sichuan, China where is calcareous purplish paddy soil site (Gu et al. 2017). Continuous fertilizer application in paddy field for 50 years which had influenced on bacteria community using pyrosequencing (Ahn et al. 2012).

As mentioned above, the applications of chemical, organic fertilizers or combined fertilizers were practical to the soil and changed the bacterial community. A report investigated the dynamics of the microbial community in the Ayutthaya soil series (Ay) that were added with silver nanoparticles using the automated ribosomal intergenic spacer analysis (ARISA) method (Chunjaturas et al. 2014). Activity of methanogen were studied in the Ay soil series of rice fields during vegetative and reproductive phase using chemical fertilizer (Chawanakul et al. 2009). Nevertheless, the Ay soil series soil series in tropical Thailand has not been studied long-term chemical fertilizers on bacteria community using metagenomics. In the past, bacterial isolation was conducted in a laboratory environment, leading to the cultivation of less than 2% of the total bacteria present (Wade et al. 2002). The application of metagenomics unveiled the bacterial diversity within the environment, offering valuable insights pertinent to agriculture, the food industry, and medicine (Bashir et al. 2014). The Ay soil series in Nakhon Pathom, Thailand are representative of the study in the current research. These soils are classified as very-fine, mixed, active, acid, isohyperthermic, and Vertic Endoaquepts. Soil characteristics and properties were revealed as very deep soil, dark gray clay, and moderately acidic (pH 6.0). The upper subsoil is clay, gray-brown, or gray-brown with red dots. The soil reaction was very acidic (pH 5.5) and rice straw yellow dots were found at a depth of 100-150 cm. The soil contained sulfur (Land Development Department 2023). The aim of the current research was to investigate the bacteria community using metagenomics and the correlation between the community and soil chemical properties in a long-term chemical fertilization of 8 years of a field experiment site of Ay soil series in Nakhon Pathom, Thailand. Our hypothesis is specific taxa abundances, are found in the unique area. The community of soil bacteria plays an important role in promoting rice growth through the decomposition of organic matter and nutrient cycling. The results may be helpful in identifying particular bacteria for use as biofertilizers and serve as a guideline for selecting fertilizers for sustainable soil fertility management.

MATERIALS AND METHODS

Study site and soil sampling. The soil was sampled before rice planting from a paddy field during the dry season after the soil was tilled under saturated or flooded conditions in Bang Phasi sub-district, Bang Len district, Nakhon Pathom province, Thailand (14.044116N and 100.237599E). General information in the area indicated that farmers used chemical fertilizer 46-0-0 at the rate of 2.67 kg/ha at 15-20 days after rice planting, 16-20-0 at the rate of 4 kg/ha at 1 and a half months after rice planting, and 18-8-8 at the rate of 4 kg/ha at 2 months after rice planting continuously for 8 years. The soil samples were taken in May 2022 before the next crop. A shovel was used to collect the topsoil at a depth of about 15 cm. Soil samples were collected once from 10 random points across an area of 16,000 square meters and then mixed on a canvas. The soil sample was divided into 4 parts, from which approximately 1 kg of soil was collected for analysis in 3 replicates. The soil samples were analyzed for soil chemical properties and DNA was extracted for metagenomics analysis.

Soil chemical properties. The analysis process commenced by weighing 20 g of soil sample mixed with 20 mL of distilled water in a 100 mL beaker to determine the potential of hydrogen (pH). A soil sample (400–500 g) was added with water until the soil was saturated and the electrical conductivity (EC) was determined (Beck 1999; Bower and Wilcox 1965; Jackson 1958). The pH and EC were measured using pH meter (Ohaus, ST3100) EC meter (Metter, FiveEasy F30), respectively. The organic matter (OM) content and available phosphorus were analyzed (Walkley and Black 1934; Bray and Kurtz 1945). Exchangeable potassium (K), calcium (Ca) and magnesium (Mg) were analyzed by the leaching method with 1 M ammonium acetate (Pratt 1965). Total nitrogen (total N) was calculated based on multiplying the organic matter content by 0.05 (Ongprasert 1992) based on the OM in the soil containing about 5% of N.

Bacterial diversity in paddy field. DNA was extracted from 250 mg soil samples using a NucleoSpin® soil kit (Macherey-Nagel, Germany) according to the manual for DNA extraction. The DNA bands was checked using 1% agarose gel. A sample of gel powder (48 mg) was placed in a 125 mL flask and added with 40 mL of 1X TAE buffer and heated in a microwave until it was completely dissolved. After the gel had been warmed, 4 µL of RedSafe nucleic acid dye (iNtRON, Korea) was poured into the gel in a tray until it had solidified. DNA samples (5 µL) were mixed with 6X dye loading dye (1.5 µL), put into the wells of the gel through 50 V for 40 min. then the DNA bands were observed on the gel under ultraviolet light. The DNA concentration was measured using Nanodrop (Maestro; Taiwan) before metagenomics analysis.

Library of the metagenomics data was prepared for Illumina MiSeq. Amplified regions of the 16S rRNA gene V3-V4, 400-450bp were processed with the barcode. All polymerase chain reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs; USA). A pipeline for bioinformatic data analysis was performed (supplementary file S1). Raw data obtained by sequencing were merged pair-end reads for each sample using FLASH (V 1.2.7) and quality filtering on the raw tags were performed to obtain the high-quality clean tags (Bokulich et al. 2013; Magoč and Salzberg 2011) according to the QIIME (V 7.1.0) quality-controlled process (Caporaso et al. 2010). Then the effective tags finally obtained. Parallel-meta tool was executed on FASTQ files followed by mapping on the SILVA database for species annotation at each taxonomic rank (Quast et al. 2013). The phylogenetic relationship of all operational taxonomic units (OTUs) representative sequences using the MUSCLE (V 3.8.31) was obtained (Edgar 2004). All sequences have been clustered into OTUs based on their 97% sequence similarity. Alpha-diversity was applied in analyzing the complexity of biodiversity for a sample based on six indices: observed-species, Chao1, Shannon, Simpson, abundance-based coverage estimator (ACE) and good-coverage.

Correlation and statistical analysis. The Pearson correlation coefficient was used to test the relationships between bacterial communities from the paddy field and soil chemical properties using Minitab V16.2.0 (Minitab L.L.C., Australia).

RESULTS AND DISCUSSION

Chemical properties of soil samples. The soil had a pH of 6.44 (slightly acidic) and electrical conductivity was 0.21 dS/m (not salty). The percentage of organic matter was very high (5.38%). The total nitrogen content was 0.27% and the available phosphorus was 16.77 mg/kg (quite high). Potassium content was 144.3 mg/kg (very high). Exchangeable calcium and magnesium were 13.70 and 6.10 cmol/kg, respectively, which were high. This soil analysis results were compared with the criteria of the Land Classification Division and FAO Project Staff (1973).

Soil bacterial community diversity. Basic information on three replications of soil from paddy field was collected to construct OTUs. The summarized data are shown in Figure 1, including the average of total tags (111,327), taxon tags (99,358), unclassified tags (59), unique tags (11,909), and OTUs taqs (3,604). The Y1-axis titled tags number means the number of tags; total tags means the number of effective tags; taxon tags means the number of annotated tags; unclassified tags means the number of unannotated tags; unique tags means the number of tags with a frequency of 1 and only occurs in one sample. The Y2-axis titled OTUs numbers means the number of OTUs to identify the numbers of OTUs in different samples. The bacterial sequences OTUs were clustered into based on 97% sequence similarity (supplementary file S2). Metagenomics data revealed bacterial diversity in the paddy field (Fig. 2).

The top-five phyla were the Proteobacteria (29.68%), Epsilonbacteraeota (21.67%), Nitrospirae (13.68%), Acidobacteria (9.30%) and Chloroflexi (7.13%), respectively. The top-five families were the Thiovulaceae (21.58%), Anaerolineaceae (3.68%), Hydrogenophilaceae (3.02%), Solibacteraceae (Subgroup_3; 2.60%) and Pedosphaeraceae (2.50%), respectively. The top-five genera were *Sulfuricurvum* (21.58%), *Thiobacillus* (2.84%), *Geobacter* (2.27%), *Anaerolineaceae*-uncultured (1.66%) and *Gemmatimonadaceae*-uncultured (0.88%), respectively. In addition, several other types of bacteria were found that could be classified or cultured. In average total, 3,444.33 genera were observed. The Shannon, Simpson, Chao1 and ACE indices indicated there was high bacterial diversity in the paddy field (Table 1).

Table 1. Diversity indices of bacteria in paddy field

Parameter	Value
Number of observed species	3,444.33 ± 326.05
Shannon index	8.30 ± 0.91
Simpson index	0.95 ± 0.05
Chao1 index	3577.56 ± 307.00
ACE index	3624.30 ± 316.55
Goods coverage index	1.00 ± 0.00

ACE = abundance-based coverage estimator

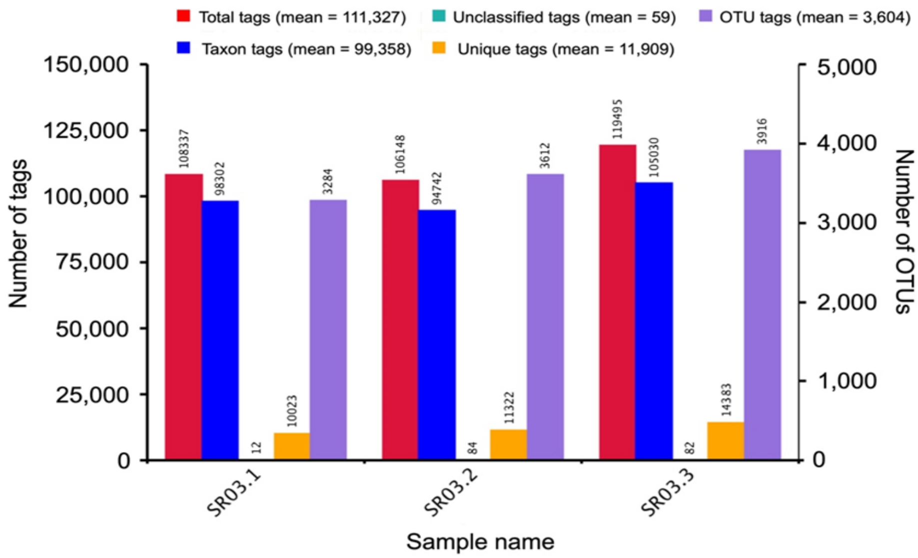

Fig. 1. Summary of numbers of tags and operational taxonomic units (OTUs) from three paddy field soil replicates (SR03.1 SR03.2 and SR03.3)

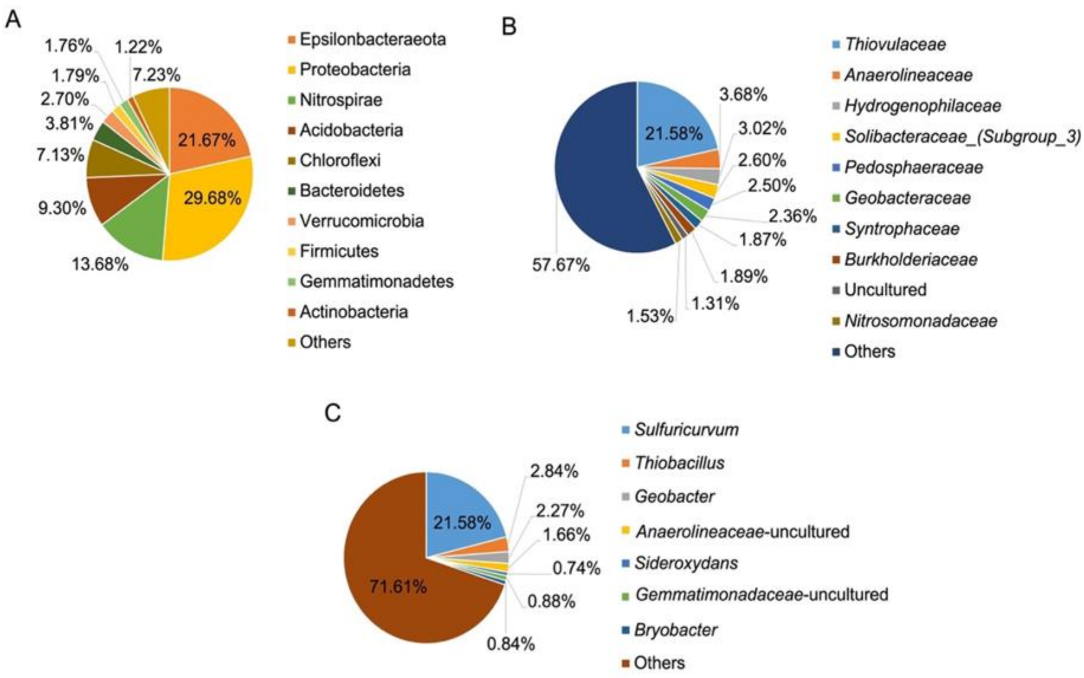

Fig. 2. Relative abundances of bacterial diversity in paddy field at levels of: (A) phylum; (B) family; (C) genus

Correlation between soil properties and microbial groups. The correlations between bacterial community from paddy fields and soil chemical properties are shown in Table 2. The criteria for separating correlation levels followed Ratner (2009). The pH was strongly positive correlated with *Sulfuricurvum* (0.95), *Thiobacillus* (0.74) and *Sideroxydans* (0.96) and strongly negative correlated with *Geobacter* (-0.83), *Anaerolineaceae*-uncultured (-0.98) and *Bryobacter* (-1.00). However, *Gemmatimonadaceae*-uncultured (-0.37) showed a moderate negative correlation. The EC had a strong positive correlation with *Sulfuricurvum* (0.74) and *Sideroxydans* (0.73) and a moderate positive correlation with *Gemmatimonadaceae*-uncultured (0.62). The EC had a strong negative correlation with *Geobacter* (-0.90), a weak negative correlation with *Thiobacillus* (-0.22) and *Anaerolineaceae*-uncultured (-0.30) and a moderate negative correction with *Bryobacter* (-0.45). The OM exhibited strong positive correlations with *Geobacter* (0.81), *Anaerolineaceae*-uncultured (0.98), and *Bryobacter* (1.00) while it was strongly negatively corrected with *Sulfuricurvum* (-0.94), *Thiobacillus* (-0.75) and *Sideroxydans* (-0.95). Similar to the total N, there were strong positive correlations with *Geobacter* (0.72), *Anaerolineaceae*-uncultured (1.00), and *Bryobacter* (0.99). However, there were strong negative correlations with *Sulfuricurvum* (-0.89), *Thiobacillus* (-0.84), and *Sideroxydans* (0.90). *Gemmatimonadaceae*-uncultured showed a moderate positive correlation with both the OM and total N (0.40 and 0.52, respectively).

Available phosphorus was strongly positively correlated with *Thiobacillus* (0.95) but weakly positively correlated with *Sulfuricurvum* (0.21), *Geobacter* (0.08) and *Sideroxydans* (0.23). The available phosphorus was strongly negatively correlated with *Gemmatimonadaceae*-uncultured (-0.99) and moderately negatively correlated with *Anaerolineaceae*-uncultured (-0.67) and *Bryobacter* (-0.55). Exchangeable potassium, calcium, and magnesium displayed strong positive correlations within a range with *Sulfuricurvum* (0.95-0.97), *Thiobacillus* (0.7-0.74), and *Sideroxydans* (0.96-0.97) but were strongly negatively correlated with *Geobacter*, *Anaerolineaceae*-uncultured and *Bryobacter*. There was a moderate negative correlation of the exchangeable potassium calcium and magnesium with *Gemmatimonadaceae*-uncultured (-0.33, -0.37, and -0.37, respectively).

Table 2. Correlation coefficient between paddy field bacterial community and soil chemical properties

	Soil property							
Genus	**pH**	**EC**	**OM**	**Total N**	**P**	**K**	**Ca**	**Mg**
Sulfuricurvum	0.95	0.74	-0.94	-0.89	0.21	0.97	0.95	0.95
Thiobacillus	0.74	-0.22	-0.75	-0.84	0.95	0.70	0.74	0.74
Geobacter	-0.83	-0.90	0.81	0.72	0.08	-0.85	-0.83	-0.83
Anaerolineaceae-uncultured	-0.98	-0.30	0.98	1.00*	-0.67	-0.97	-0.98	-0.98
Sideroxydans	0.96	0.73	-0.95	-0.90	0.23	0.97	0.96	0.96
Gemmatimonadaceae-uncultured	-0.37	0.62	0.40	0.52	-0.99	-0.33	-0.37	-0.37
Bryobacter	-1.00*	-0.45	1.00*	0.99	-0.55	-0.99	-1.00*	-1.00*

pH = potential of hydrogen, EC = electrical conductivity, OM = organic matter, Total N = total nitrogen, P = phosphorus, K = exchangeable potassium, Ca = calcium and Mg = magnesium.

Minitab (version 16.2.0; Minitab, L.L.C., Sydney, two letter state abbreviation if applicable, country) statistical software was used for correlation testing and significant ($p < 0.05$) differences are indicated as *.

Bacterial diversity and bacterial relationships with soil chemical properties were studied in paddy fields of Ay soil series in Nakhon Pathom, Thailand, which underwent long-term chemical fertilization of 8 years. The soil analysis indicated that the soil was fertile, with high diversity of microbial communities and the dominant bacteria were in the phylum Proteobacteria. The gamma-Proteobacteria was sensitive to all the fertilization regimes in paddy soil as affected by long-term application of inorganic fertilizer and rice straw using 16S rRNA (Wu et al. 2011). The Proteobacteria Chloroflexi, and Acidobacteria phyla were found dominant in rice paddy soil with different fertilizers management for a period of 50 years (Ahn et al. 2012). The relative abundance of Nitrospirae and Gemmatimonadetes phyla changed when using a 30-year organic-inorganic fertilization in a paddy field (Yang et al. 2019).

Notably, the phylum of Epsilonbacteraeota was predominant in the current research, especially the family, *Thiovulaceae* accompanied by the genus *Sulfuricurvum*. Previous studies showed the abundance of *Sulfuricurvum* in different fertilization regimes. The relative abundance of *Sulfuricurvum* was high following the simultaneous application of long-term inorganic fertilizers and rice straw compost, they are a group of chemoautotrophs bacteria found in paddy soil and paddy sediment that can oxidize sulfur and sulfur compounds with oxygen or nitrate as an electron acceptor (Ahn et al. 2012; Jiang et al. 2021).

Thiobacillus and *Geobacter* were also found in the current research, which is similar to the report of Ahn et al. (2012). *Thiobacillus* and *Sideroxydans* are important genera in the phylum Betaproteobacteria a group of bacteria chemoautotrophs that use iron or compound sulfur as an energy source. *Thiobacillus* species play an important role in the oxidization of sulfur, decrease the soil pH, make soil conditions for plant growth and help plants with nutrient absorption (Akhtar et al. 2012).

The presence of *Sulfuricurvum*, *Thiobacillus*, *Geobacter* and *Nitrospira* in rice growth and reproductive stages improved crop production and health (Baskaran et al. 2022). The iron-reducing bacteria *Geobacter* and *Anaeromyxobacter* genera were also recently reported as nitrogen-fixing bacteria predominant in paddy soils (Masuda et al. 2021). Long-term application of organic fertilizer or combined with inorganic fertilizer, improved soil quality, and environmental safety by increasing the number of methane-oxidizing bacteria and nitrogen-fixing bacteria that are beneficial to the soil and increasing crop yields (Daquiado et al. 2016).

The current research showed the total nitrogen content was significantly positively correlated with the genus *Anaerolineaceae*-uncultured. The *Anaerolineaceae* significantly increased by long-term use of inorganic fertilizers over a 19-year period on increasing functions and bacterial diversity in Chinese paddy field soils (Huang et al., 2019). *Anaerolineaceae* and other bacteria were correlated with various metabolic pathways and soil properties in paddy soil from a rice-wheat cropping system over a 10-year period using 4 treatments: control, NPK, NPK + pig manure, and NPK + straw (Wang et al. 2019). These bacteria are found in sedimentary environments and are involved in the degradation of organic nitrogen (Yamada et al. 2006).

Organic matter was significantly positively correlated with the *Bryobacter* genus which is in the phylum Acidobacteria of the current research. Soil organic matter determines the activity of Acidobacteria when applied long-term chemical fertilizer (Ahn et al. 2016). The relative bacterial abundances of *Geobacter and Bryobacter* also increased when applied long-term chemical fertilization and crop rotation (Chen et al., 2020). The phyla Proteobacteria, Acidobacteria, Chlorobi, and Bacteroidetes were correlated with the available potassium, available phosphorus, and soil organic matter (Zhu et al. 2019). The bacterial phyla Acidobacteria, Gemmatimonadetes, and Cyanobacteria were significantly correlated with soil organic matter and/or nitrate contents (Chen et al. 2016). *Bryobacter* are heterotrophic bacteria that contribute to the degradation of organic molecules (Kulichevskaya et al. 2010; Thouin et al. 2019). It is an indicator that degrading bacterial communities in rice paddy fields subsidize the carbon pool (Xuan 2012). These mentioned bacteria are associated with soil properties and can be isolated for use as biofertilizers in rice cultivation, thereby reducing the dependency on chemical fertilizers.

CONCLUSION

The paddy field soil of the Ayutthaya soil series in Bang Len district, Nakhon Pathom province, Thailand was fertile with a high level of organic matter. The soil had 8 years of long-term application of chemical fertilizers only which resulted in specific bacterial taxa. The prominent genus present was *Sulfuricurvum*. There was high bacterial diversity that was correlated with various soil properties. In addition, specific group of sulfur-oxidizing, iron-reducing, nitrogen-fixing, and nutrient-solubilizing bacteria taxa were found. Therefore, these bacteria can be isolated and applied together to promote rice growth.

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ATTRACTIVENESS AND TOXICITY OF IMIDACLOPRID AND DELTAMETHRIN INSECTICIDES AGAINST *Tetragonula laeviceps* SMITH (Hymenoptera: Apidae: Meliponini)

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ABSTRACT

Tetragonula laeviceps is one of the bees that plays an essential role in pollinating plants. Currently, the existence of bee populations is in danger of decreasing. One of the reasons was the inappropriate use of pesticides. The pesticide groups that influence bee behavior and mortality are neonicotinoid and pyrethroid. This study sought to determine the attractiveness and toxic effect of imidacloprid (neonicotinoid) and deltamethrin (pyrethroid) insecticides on *T. laeviceps*. The experiments were conducted on May to July 2022 at Insect Physiology and Toxicology Laboratory, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University. The olfactory response of *T. laeviceps* was carried out using a Y-tube olfactometer with odor sources including imidacloprid (200 ppm), deltamethrin (12.5 ppm), mineral water and honey. Toxicity bioassays of *T. laeviceps* were carried out by topical, residual, and oral applications at five insecticide concentrations. Bees were more attracted to honey and water than imidacloprid 200 ppm or deltamethrin 12.5 ppm, while the attractiveness between imidacloprid 200 ppm and deltamethrin 12.5 ppm did not show a significant difference. The toxic effect of imidacloprid on *T. laeviceps* was greater than deltamethrin for the three different bioassays of insecticides. These two active ingredients were classified as highly toxic (LD_{50} <2 μg/bee) based on EPA classification. The implication of these results is the farmer should not spray the insecticide particularly imidacloprid and deltamethrin on their chili plantation during flowering stages.

Key words: mortality, olfactometer, oral application, residual application, topical application

INTRODUCTION

Most plant species are pollinated by insects (Michener 2007). Roughly one-third of the food is directly or indirectly dependent upon pollination by bees (Peters 2013). One of the native bees in Indonesia is *Tetragonula laeviceps* Smith (Apidae: Meliponini). *T. laeviceps,* known as stingless bee is domesticated by beekeepers as friendly bee because it has no sting. *T. laeviceps* is used to produce honey, pollen and propolis. In addition, *T. laeviceps* can also be used as a plant pollinating agent (Putra et al. 2014). *T. laeviceps* bees actively collect nectar and pollen from various types of plants (Cholis et al. 2019). Cross-pollination by bees significantly increases the quantity and quality of yield in plants (Stein et al. 2017). Plants pollinated by bees have an increased number of seeds and fruit size. Some types of plants, such as cucumber, chili, and tomato, increase fruit size and the number of seeds when pollinated with the help of bees (Zidni et al. 2021; Mubin et al. 2022a).

Pollination by bees contributes to more than half of the US agriculture industry's value of $29 billion per year (EPA 2018). Despite bees' importance, bee colonies' populations have declined in

Europe and North America. Scientists have identified many factors influencing this decline, such as diseases and bee pests, poor bee nutrition, lack of genetic diversity, bee management practices, pesticide use (EPA 2018) as well as colony collapse disorder (CCD) phenomenon (Lu et al. 2012). Some insecticides that are thought to reduce the presence of bee colonies are neonicotinoid such as imidacloprid and pyrethroid such as deltamethrin. Exposure to imidacloprid results in bees experiencing disorientation and lose direction to returning to the hive or their food source (Yang et al. 2008). In addition, bees' learning and memory abilities will generally be impaired in terms of the ability to find and navigate due to exposure to imidacloprid (Zhang and Nieh 2015). Deltamethrin can affect foraging activity and the development of bee colonies. Honey bee (*A. mellifera*) communication through waggle dance was disrupted by deltamethrin-sublethal doses (Zhang et al. 2019). Exposure to the imidacloprid and deltamethrin insecticides disrupted bee activity in foraging for food (Tan et al. 2014; Zhang et al. 2019). The loss of bees will cause a massive problem for the agricultural ecosystems such as chili, tomato, cucumber, melon, citrus, coffee and others. Therefore, this study sought to determine the attractiveness and toxic effect of imidacloprid (neonicotinoid) and deltamethrin (pyrethroid) insecticides on *T. laeviceps*.

MATERIAL AND METHODS

Study site. This research was carried out at the Insect Physiology and Toxicology Laboratory, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University from May to July 2022.

Experimental materials. The *T. laeviceps* worker bees used were obtained from beekeepers in Sumedang, West Java-Indonesia. Olfactory and toxicity bioassays were carried out using commercial insecticide products of imidacloprid (5%) and deltamethrin (25 g/l).

Olfactory bioassay. Olfactory bioassay was used to evaluate the attractiveness of bees to insecticides, water and honey. The bioassays can be done in several ways, such as testing with live plants, flowers, prey, or other aroma. In this experiment, the attractiveness of insecticide aroma was evaluated. The single concentration of insecticides was used in olfactory bioassays based on the recommendation rate as shown on the pesticide labels. The concentrations used were 200 ppm and 12.5 ppm for imidacloprid and deltamethrin, respectively (Table 1). Each treatment used 15 bees (as replication) which were acclimatized for 2 hours in a gauze cage (30 cm x 30 cm x 30 cm) before treatment.

Table 1. Sources of aroma treatments for olfactory bioassay.

Sub-test	Code	Treatments
P1	IMD vs HON	Imidacloprid 200 ppm *vs* 10% aqueous honey
	DEL vs HON	Deltamethrin 12.5 ppm *vs* 10% aqueous honey
	IMD-HON vs HON	Imidacloprid 200 ppm in 10% honey solution *vs* 10% aqueous honey
	DEL-HON vs HON	Deltamethrin 12.5 ppm in 10% honey solution *vs* 10% aqueous honey
P2	IMD vs WTR	Imidacloprid 200 ppm *vs* mineral water
	DEL vs WTR	Deltamethrin 12.5 ppm *vs* mineral water
	IMD-HON vs WTR	Imidacloprid 200 ppm in 10% honey solution *vs* mineral water
	DEL-HON vs WTR	Deltamethrin 12.5 ppm in 10% honey solution *vs* mineral water
P3	IMD-HON vs DEL-HON	Imidacloprid 200 ppm in 10% honey solution *vs* Deltamethrin 12.5 ppm in 10% aqueous honey solution
	IMD vs DEL	Imidacloprid 200 ppm *vs* Deltamethrin 12.5 ppm

Description codes: IMD: Imidacloprid 200 ppm, DEL: Deltamethrin 12.5 ppm, HON: 10% aqueous honey, WTR: mineral water, IMD-HON: Imidacloprid 200 ppm in 10% honey solution, DEL-HON: Deltamethrin 12.5 ppm in 10% honey solution

Olfactory bioassays were carried out using a Y-tube olfactometer (Li et al. 2014). Each test solution was pipetted (200 µl) on a piece of foam (2 cm x 2 cm x 2 cm) which was placed inside a small tube (5 cm diameter; 10 cm height) connected to the arm end of the Y-tube olfactometer. The top of the glass tube was covered with gauze and the arm of the Y-tube olfactometer was attached to the mini vacuum pump. Every 20 minutes, the test solution was applied again to reduce loss from evaporation. A flowmeter was installed between the olfactometer and the pump to maintain an air flow rate of 40 ml/min. One *T. laeviceps* bee was placed at the end of the olfactometer arm, and observed for 10 minutes. The olfactometer was used only once, after which it was cleaned and dried for further use.

Toxicity test. Toxicity tests were carried out by topical, residual, and oral applications.

Topical application. The test concentrations of imidacloprid (a.i.) were 200, 20, 2, 0.2, 0.02 ppm, while the concentrations of deltamethrin (a.i.) were 187.5, 125, 12.5, 1.25, 0.125, 0.0125, 0.00125 ppm. Imidacloprid and deltamethrin insecticide formulations were diluted with acetone. Acetone was served as a control.

T. laeviceps worker bees were acclimatized before treatment by placing inside a test tube (30 mm in diameter; 200 mm in height) for one hour and anaesthetized in a refrigerator at -18°C for 3 minutes. Each concentration of insecticide (1 µl) was applied to the dorso-thorax of the bees using a micro syringe applicator (EPA 2014). Each treatment was replicated three times with 10 bees per replication. After treatment, the test bees were placed in a glass tube (10 cm in diameter, 15 cm in height) and fed with 10% honey solution. Mortality of bees was observed at 48 hours after treatment (HAT).

Residual application. The concentrations of imidacloprid tested were similar to topical application test, while the concentrations of deltamethrin as follow 375, 312.5, 250, 187.5, 125, 12.5, 1.25, 0.125, 0.0125, 0.00125 ppm. Acetone was used as solvent. The bioassay method used referred to Syahputra (2001). The test solution (500 µl) was poured inside a glass tube (30 mm in diameter; 200 mm in height), then the inner surface of the glass tube was coated evenly with the test solution by rolling the glass tube thoroughly. After that, the tube was dried until the solvent evaporated. A total of 10 bees were exposed to each treated glass tube for 5 minutes, transferred into a fresh rearing tube and fed with 10% aqueous honey solution absorbed in a cotton swab. Each treatment was replicated three times. Bee mortality was observed at 48 HAT.

Oral application. The concentrations of imidacloprid tested were similar to topical application test, while the concentrations of deltamethrin as follow 1250, 375, 312.5, 250, 187.5, 125, 12.5, 1.25, 0.125, 0.0125, 0.00125 ppm. The insecticides were diluted with 50% aqueous honey solution. Ten *T. laeviceps* were fasted for 1 hour before treatment in tube (10 cm in diameter, 15cm in height). Then, the bees were treated with each concentration of treatment by placing cotton swab containing each concentration of insecticides at the top of the tube. 50% aqueous honey solution was served as control. The bees were let to fed for 5 minutes. After that, all the cotton swabs containing insecticides were removed and then changed to untreated cotton swabs. *T. laeviceps* bees that had been treated were reared and fed with 10% honey solution absorbed in cotton. Each treatment was replicated three times. Mortality observations were made at 48 HAT.

Data analysis. The attractiveness data were analyzed by chi-square test using the Rstudio program. The bee mortality data were analyzed by probit analysis using the PoloPlus program to determine LD_{50} or LC_{50} values. The LD_{50} value was used for the classification of the toxicity value of the test substance on bees according to the US EPA (2014) (Table 2).

Table 2. Toxicity classification to bees based on LD_{50}

LD_{50} (μg/bee)	**Toxicity classification**
≥ 11	Non-toxic
11 > LD_{50} > 2	Moderately
≤ 2	Highly toxic

Source: US EPA (2014)

RESULTS AND DISCUSSION

Attractiveness of imidacloprid and deltamethrin. Bees rely heavily on their sense of smell (olfactory) for various aspects of their lives, one of which is when bees are looking for food (Paoli and Galizia 2021). *T. laeviceps* spent significantly more time in the arm connected to the honey source over imidacloprid 200 ppm and deltamethrin 12.5 ppm (Fig. 1). There was significant difference in the attraction of *T. laeviceps* for honey compared to imidacloprid 200 ppm and deltamethrin 12.5 ppm (p-value <0.01). This implies that imidacloprid 200 ppm and deltamethrin 12.5 ppm did not disturb the bees visiting behavior for honey (nectar).

Olfactometric response of *T. laeviceps* to mineral water (P2) was higher than response to either imidacloprid 200 ppm, deltamethrin 12.5 ppm or deltamethrin 12.5 ppm in 10% honey solution (p-value <0.05). However, imidacloprid 200 ppm in 10% honey solution did not show significant difference with the mineral water treatment (Fig. 1).

All P3 treatments showed that the attractiveness of imidacloprid 200 ppm and deltamethrin 12.5 ppm was not significantly different with or without honey mixtures (Fig. 1). The average percent attraction of *T. laeviceps* to the scent of imidacloprid 200 ppm was 53.3% compared to 46.7% for deltamethrin 12.5 ppm (P3).

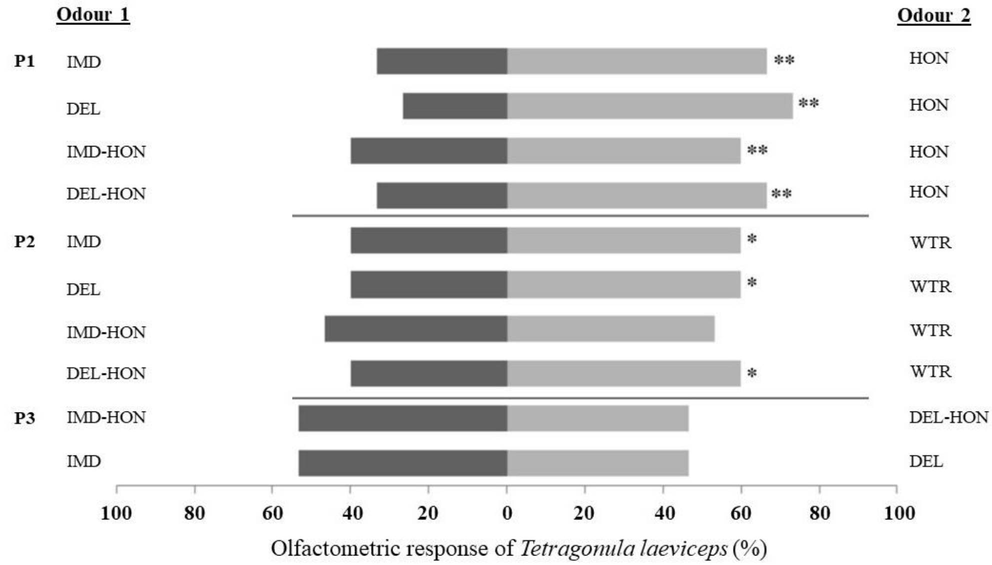

Fig. 1. The olfactometric response of *Tetragonula laeviceps* in the test using a Y-tube olfactometer with two aroma sources. (P1) insecticide -*vs*- honey, (P2) insecticide -*vs*- mineral water, (P3) imidacloprid -*vs*- deltamethrin.**= p-value < 0.01, *= p-value < 0.05 X^2 test. Description: IMD: Imidacloprid 200 ppm, DEL: Deltamethrin 12.5 ppm, HON: 10% aqueous honey, WTR: mineral water, IMD-HON: Imidacloprid 200 ppm in 10% honey solution, DEL-HON: Deltamethrin 12.5 ppm in 10% honey solution

The mixture of insecticide preparations with honey illustrates the field conditions when bees can still visit plants or flowers that have been exposed to insecticides. *Apis mellifera* and *Bombus terrestris* had a higher preference for sucrose solution containing imidacloprid and thiamethoxam than 0.5 M sucrose solution alone under constant temperature and 60% RH (bumblebees: 28 °C; honeybees: 34 °C) (Kessler et al. 2015). Honey and water are necessary for the life of *T. laeviceps* bees. Bees need honey as food and water to regulate the temperature in the hive. Bees consume processed nectar (honey) which provides energy for flight, colony maintenance, and general daily activities (Ellis et al. 2020), while water is used by bees to obtain mineral content and to regulate temperature conditions (thermoregulation) inside the hive when conditions are hot outside the hive (Stabentheiner et al. 2021).

Mortality of *T. laeviceps*. Bees can still visit plants or flowers that have been sprayed with pesticides to obtain food sources such as nectar and pollen to meet the colony's needs. As a result, bees can be exposed to pesticides through contact and oral exposures. Toxicity of imidacloprid against *T. laeviceps* in the three toxicity test methods showed a linear relationship between the concentration of the active ingredient and bee mortality (Fig. 2). 100% mortality was reached at 2 ppm for topical application, while oral and residual applications need higher concentration (20 ppm). These concentrations were far below the recommended spraying concentration (200 ppm).

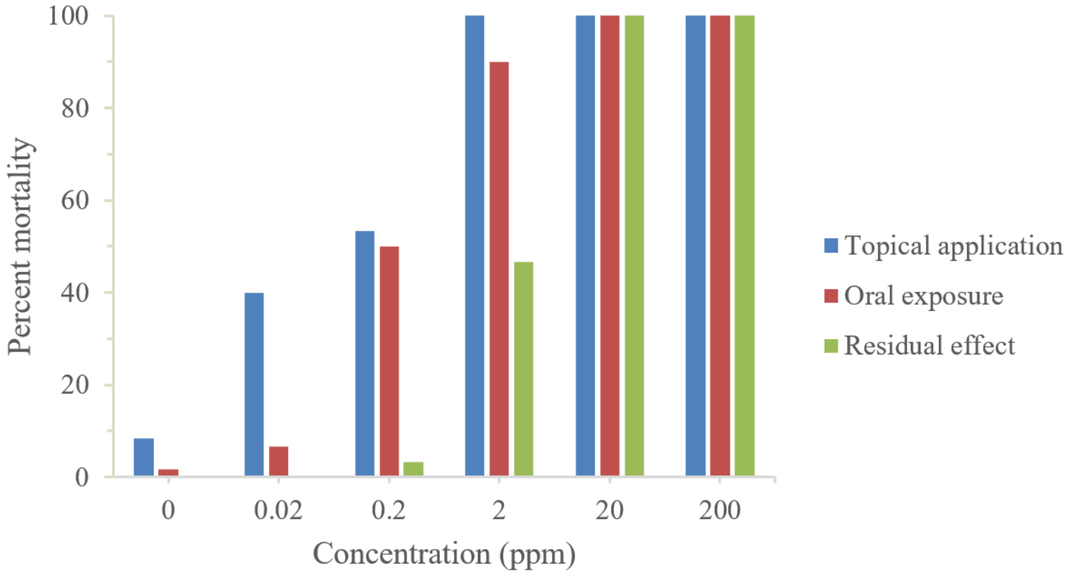

Fig. 2. Toxicity of imidacloprid to *Tetragonula laeviceps*

The exposure of *T. laeviceps* to deltamethrin by the topical application also showed a linear relationship between the concentration of the active ingredient and bee mortality, from 0.00125 to 187.5 ppm deltamethrin (Fig. 3). However, in oral application method, percent of bee mortality increased until 312.5 ppm and then decreased at higher concentrations (375 ppm). The bees stayed away from the test feed containing the insecticide deltamethrin at 375 ppm. The avoidance indicated there was a repellent effect, so bee mortality was lower when the concentration of deltamethrin insecticide reached 375 ppm (Fig. 3).

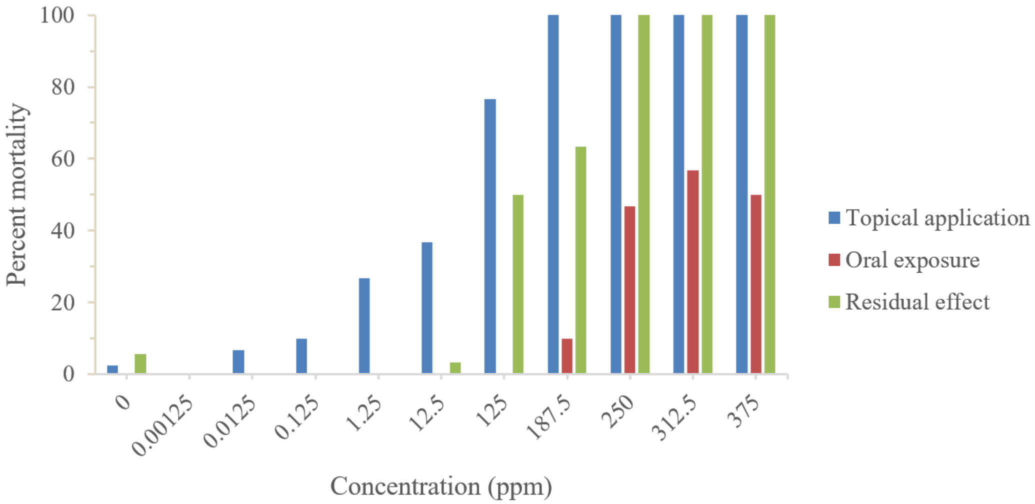

Fig. 3. Toxicity of deltamethrin to *Tetragonula laeviceps*

The mortality tests also proved the disinterest of *T. laeviceps* to deltamethrin aroma at high concentrations (375 ppm) compared to the recommended concentration (12.5 ppm). Based on the results of these tests, *T. laeviceps* was more attracted to the aroma of deltamethrin at 12.5 ppm (p-value <0.05) in five minutes (Fig. 4). It seems that high concentration of deltamethrin (375 ppm) in oral test repelled *T. laeviceps*.

Deltamethrin at concentration 0.52% has a repellent effect on *Aedes aegypti* mosquitoes, so visiting decreased (Bowman et al. 2018). A decrease in foraging of *Apis mellifera* was observed for permethrin at 8 µg/cm^2 and 4.45 µg/cm^2 (filter paper) in flight cage bioassays of bees (Rieth and Levin 1988; Ingram 2013). Deltamethrin and permethrin are an insecticide of the pyrethroid class that acts as contact and stomach poisons (NPIC 2010).

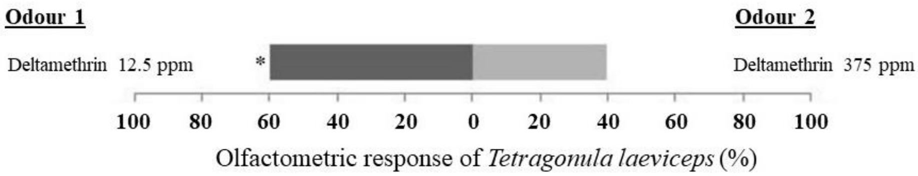

Fig. 4. The olfactometric response of *Tetragonula laeviceps* in the test using a Y-tube olfactometer between deltamethrin 12.5 ppm and deltamethrin 375 ppm aroma sources. *= p-value < 0.05 X^2 test.

Deltamethrin at low dosage (5 g/ha) was used on pollen beetle of oilseed rape, but deltamethrin had a repellent effect on honey bees (Bos and Mason 1983). Although deltamethrin has a repellent effect on bees, it does not mean that it is safe for bees. The repellent effect of pyrethroid compounds did not protect bees visiting plants after application (Rieth and Levin 1988). The repellent effect of pyrethroid compounds is estimated to last about 24 hours, so it is not long enough to prevent bees from residual contact toxicity (Rieth and Levin 1988). After contact with the pyrethroid insecticide, the bees returned to the colony and were inactive for a period ranging from less than 1 hour to a maximum of 24 hours. After recovery, normal foraging resumed and the bees continued foraging at the same site to which they were previously conditioned (Rieth and Levin 1988). At that time, the bees came in contact with the insecticide residues, demonstrating its high toxicity to bees in these topical and residual exposure.

Bees were generally more intolerant to contact (topical) application than oral or residual application. Imidacloprid (0.02 ppm) produced 40% mortality to bees by topical application, while deltamethrin (0.0125 ppm) produced 6.7% mortality (Fig. 2 and 3). The contact toxicity of imidacloprid was generally highly toxic to the *Bombus impatiens*, *Osmia lignaria*, and *Megachile rotundra*; while deltamethrin was less toxic (Scott-Dupree et al. 2009). Treatment of imidacloprid at 0.125 ppm by oral application caused high mortality (more than 45%) to *A. mellifera* (Sanchez-Bayo et al. 2017), while in this study, imidacloprid caused 50% mortality at 0.2 ppm.

Bioassays using the topical application method simulates exposure of bees to insecticides in the field (Fig. 5a). The bees treated by topical application will be exposed to insecticides much more than oral and residual applications at the same concentration so that the mortality of *T. laeviceps* treated by topical application was higher than two other methods.

Honey bee (*A. mellifera*) and stingless bee (*T. laeviceps*) are pollinators, foraging for food to feed the colony. Food source (nectar) that are exposed to insecticides will have a negative impact on bees.

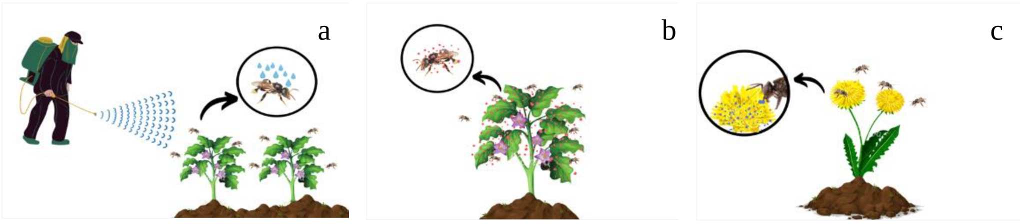

Fig. 5. A simple conceptual model for pesticide exposure in the field based on mortality testing with topical (a), residual (b), and oral application (c).

Bees exposed to imidacloprid dermally (topical and residual) or orally will die. However, other possible impacts of insecticides can harm the bee colony. When bees bring nectar or pollen contaminated with slow-acting insecticides to their hives, the entire colony will be exposed to insecticides (Traynor et al. 2021; Mubin et al. 2022b). The recommended dose of insecticides used in this study could kill the test bees. In addition, sublethal doses of imidacloprid and deltamethrin will reduce foraging activities and other activities at the hive entrance of *Apis mellifera ligustica* in field tests (Decourtye et al. 2004). The exposure of the whole bee colony is caused by trophallactic behavior or the feeding behavior of other colony members (breeding nests) (Gernat et al. 2018).

Toxicity of imidacloprid and deltamethrin to *T. laeviceps*. The toxicity of imidacloprid and deltamethrin insecticides against *T. laeviceps* varied for each type of exposure (Table 3). In general, imidacloprid was more toxic than deltamethrin as shown by the lower LC_{50} values.

Table 3. LC_{50} of imidacloprid and deltamethrin insecticides against *Tetragonula laeviceps*.

Active ingredient	LC_{50} (ppm)		
	Topical application	Residual application	Oral application
Imidacloprid	0.08	1.92	0.23
Deltamethrin	19.08	141.76	279.32

The insecticide imidacloprid acts as a competitive modulator of the nicotinic acetylcholine receptor (nAChR). Imidacloprid binds to nicotinic acetylcholine receptors causing continuous stimulation while deltamethrin works as a sodium channel modulator (IRAC 2022). Deltamethrin will slow down sodium channel closure. Both insecticides will cause the exposed bees to experience convulsions, paralysis, and eventually die.

Although deltamethrin was less toxic than imidacloprid, both insecticides were classified as harmful insecticides to bees (Decourtye et al. 2004). Imidacloprid and deltamethrin could cause a decrease in the activity of *A. mellifera* in foraging (Telangre et al. 2018; Dworzanska et al. 2020). *A. mellifera* and *B. terrestris* sometimes had less active bees than the unsprayed control in deltamethrin plots sprayed, but thiacloprid did not have any tendency to be repellent (Havstad et al. 2019). Deltamethrin interferes with the nervous system of bees, such as dance behavior. Bee workers use social learning when following the waggle dance to learn resource location and quality. Referential communication codes information and the dancer encodes the polar coordinates of a resource relative to the nest (Dong et al. 2023). Bee dance behavior (waggle dance) on bees that consume sucrose syrup with a mixture of deltamethrin insecticide were less precise than those that consumed normal sucrose syrup. The information about the direction and distance to the advertised source conveyed becomes inaccurate compared to bees that consume sucrose alone (Zhang et al. 2019). This causes bees returning to the nest to confuse other nestmates in food recruitment. Forager bees will have difficulty finding food sources from the information provided by previous workers so the pollination process will decrease due to the absence of pollinating bees on the plants.

Based on the EPA classification (2014), both LD_{50} values of contact insecticides, imidacloprid and deltamethrin, were classified as highly toxic insecticides to bees (Table 4). The most apparent effect when bees are exposed to pesticides was the death of worker bees after direct exposure (Gradis et al. 2010). However, pesticides can also cause sub-lethal effects for bees. Exposure to pesticides at sub-lethal concentrations for bees could lead to a shorter life span, behavioral changes, decreased immunity, decreased fecundity, and abnormal development. Bee production and vitality can also decrease when contaminated pollen is collected and fed (Gradis et al. 2010; Chmiel et al. 2020).

Table 4. Contact toxicity of imidacloprid and deltamethrin to *Tetragonula laeviceps* based on EPA classification.

	Imidacloprid	Deltamethrin
LD_{50} (µg/bee)	0.0098	0.038
EPA Classification*	Highly toxic	Highly toxic

* Toxicity based on US EPA (2014): LD_{50}< 2 (highly toxic); LD_{50} = 2-11 (moderately); LD_{50}> 11 (non toxic)

CONCLUSION

Water and honey (as food source) are more attractive than imidacloprid 200 ppm and deltamethrin 12.5 ppm for bees to visit the food sources of nectar and pollen. Thus, when farmers spray these two insecticides in chili plantations, the bees are exposed as these are exploring and looking for food sources. However, imidacloprid and deltamethrin are highly toxic to bees, both orally and dermally. Contact of bees to contaminated nectar will lead to bee mortalities, decrease in bee population and less production of cultivated plants. This information is crucial for beekeepers and chili farmers, in particular, so spraying of both insecticides should be avoided during the flowering stage of chili.

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QUANTIFICATION OF TOTAL PHENOLIC AND TOTAL FLAVONOID COMPOUNDS IN SWEET BASIL (*Ocimum basilicum* L.) LEAVES, THROUGH THE OPTIMIZATION OF TEMPERATURE AND CONCENTRATION OF ETHANOL

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ABSTRACT

Sweet basil (*Ocimum basilicum* L.) is a valuable pharmaceutical herb that is rich in antioxidants. Despite this, the success in extracting those compounds lies on the technique used during the extraction processes. The current study was aimed to establish a suitable extraction technique through different levels of temperature and concentrations of ethanol. The study was conducted in Post-Harvest Laboratory of Agriculture Faculty in Universiti Putra Malaysia, Malaysia from July 2019 to February 2020. The mature dried leaves of sweet basil were exposed to three different temperatures (40, 60 and 80°C) and combined with three concentrations of aqueous ethanol solutions (60, 75 and 90%, v/v). The experiment was carried out in 3 X 3 factorial complete randomized design with four replications and three samples per replicate. The highest extraction yield (11.56%) was obtained from the combination of 80°C + 90% aqueous ethanol. Meanwhile, the highest total phenolic content (67.02 mg of GAE/g DE), total flavonoid content (44.70 mg QUE/g DE) and antioxidant activity (66.80% in 100 µg/ml) were obtained from the combination of 80°C + 60% aqueous ethanol. Although the highest extraction yield was obtained from 80°C+90%, it is however, this study was focusing on the phenolic and flavonoid compounds. Hence, it is recommended to extract the sweet basil leaves under 80°C with 60% aqueous ethanol.

Key words: antioxidants; extraction; flavonoid; phenolic; sweet basil

INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) is known as a royal aromatic herb, as it is rich in numerous phytochemicals (Filip 2017). It is considered as a natural edible source of antioxidant compounds (Patriani et al. 2021). There are more than 200 different phytochemicals are reported in sweet basil (Ghasemzadeh et al. 2016). Among all of the phytochemicals, phenolics and flavonoids are major groups of health beneficial antioxidant compounds (Boyer and Liu 2004; Nyamai et al. 2016; King and Young 1999). These groups of compounds are widely used as food ingredients (Tanna et al. 2019).

The extraction of antioxidant compounds from plants involves the withdrawal of unseen compounds from solid particles of plant materials. Establishing methods for extraction of selected antioxidant properties is necessary to ensure high extraction yield. Conventionally, antioxidants are extracted by using methods such as Soxhlet and maceration (Arceusz et al. 2013; Gallo et al. 2020; Turrini et al. 2019). The main drawback of Soxhlet extraction is time consuming and use of large volume of pure solvent, which is not economical (Arceusz et al. 2013), while maceration is time consuming and has low efficiency (Zhang et al. 2018). Alternatively, solvent extraction method is assumed as of the most economical (in terms of time and efficiency) and easy in terms of operation. Previous researchers claimed that organic solvent extraction was an effective method for extracting phenolic compounds from plant materials, in addition to the instrument (Dorta et al. 2012; Halim 2020; Khoddami et al. 2013).

However, there are some factors involved in influencing the efficacy of extraction process. Among them, the concentration of solvent and level of temperature are the most critical factors that affected the extraction of antioxidant compounds from plant materials (Espada-Bellido et al. 2017; Najafabadi et al. 2020). There were several recommendations on the concentration of solvent to be used which ranges from 60 to 90% (v/v), depending on species (Cacace and Mazza 2003; Elboughdiri 2018; Ko et al. 2018; Toh et al. 2016; Vongsak et al. 2013; Yu et al. 2005). According to Maulana et al. (2019) and Rafińska et al. (2019), the highest amount of phenolic and flavonoid was obtained using aqueous ethanol solution at 90% and 96% in *Toona sinensis* and *Lepidium sativum* plants, respectively. Do et al. (2014) extracted antioxidant compounds from *Limnophila aromatica* with 100% ethanol recorded to receive the optimum amount of total phenolic (40.5 mg of GAE/g of DE), total content of flavonoid (31.11 mg of QUE/g of DE) with highest antioxidant activity (90%). As there is such a wide range of solvent concentrations, it is necessary to test different solvent concentrations on a given species. Similarly, different authors have reported extracting antioxidant compounds at temperature interval of 40 to 80°C (Alberti et al. 2014; Bubalo et al. 2016; Casagrande et al. 2018; Setyaningsih et al. 2019; Sumere et al. 2018; Spigno et al. 2007; Wang et al. 2013). Even for the same plant part (leaf) under same solvent concentration (75% aqueous ethanol solution), the optimum level of temperature for extraction of maximum phenolic compounds from lemon grass (*Cympopogon citratus*) and rosemary (*Rosmarinus officinal*) were 25°C and 75°C, respectively (Juntachote et al. 2006).

Overall, a few publications optimized factors such as temperature and concentration of aqueous ethanol solution for extracting antioxidants from herbal leaves and very few focused on sweet basil. For instance, Teofilović et al. (2017) suggested 96% aqueous ethanol solution and Vidović et al. (2012) established combination of 75.33ºC and 73.66% aqueous ethanol solution; however, the first one did not evaluate temperature and as for the second one, it's difficult for the industry to adjust temperature in decimal points (75.33 ºC). Therefore, this research aimed to determine the suitable temperature and concentration of aqueous ethanol solution to obtain the optimum amount of phenolics and flavonoid compounds from sweet basil leaves.

MATERIAL AND METHODS

Treatments and experimental design. Sweet basil in the form of dry leaf powder were extracted to determine the percentage of extraction yield, total phenolic content, total phenolic yield, total flavonoid content, total flavonoid yield and antioxidant activity by using different temperatures and concentrations of aqueous ethanol solution. The leaves samples were extracted at three different temperatures which were 40, 60 and 80°C and combined with three concentrations of aqueous ethanol solution which were 60, 75 and 90%, v/v. Samples were arranged in three batches and the extractions were performed for three times at 40, 60 and 80°C, respectively. This study was organized in 2-factorial Complete Randomized Design (CRD) by means of four replications and three units per replicate. The study was conducted at Postharvest Laboratory, Crop Science Department, Faculty of Agriculture, Universiti Putra Malaysia**.**

Preparation of plant materials. The leaves of sweet basil were collected from the plants at the age of 60 days after planting. Fresh leaves were separated from stems and washed to remove dust and unwanted substances. The leaves were then placed in an envelope (Manila Envelope, size: 28 cm X 33 cm), and dried using an electronic oven with air renewal circulations (Schutzart DIN 40050 – IP 20, Memmert, model: UsLM 500) at 50°C (Rocha and Melo 2011) for 7 days. Dried leaf samples were ground in fine particles using electronic grinder (Culatti, Nr. 10318194, Model: MFC) to increase the contact of sample particles with the solvent (Barros et al. 2013). The powdered samples were then placed in small plastic containers and prepared for the extraction.

Extraction. In each observation, 10 g of leaf powder sample was placed into a 250 ml conical flask and aqueous ethanol solution was added at the concentrations of 60, 75 or 90%, v/v at 100 ml for each treatment. After that, the flasks were placed in water-bath (model 760, Schutzart DIN 40050 – IP 20, Memmert, Germany) and subjected to different levels of temperatures (40, 60 and 80°C). Each temperature treatment was set for 90 minutes. The mixture was then filtered using filter pepper (Whatman No. 1, GE Healthcare UK). These procedures were repeated twice in order to make sure that all the targeted compounds were extracted from the leaves. The liquid extract from first and second extractions were combined and proceeded to the next procedure.

The liquid extract was placed in a round flask of 500 ml and placed to an Eyela rotary evaporator (Model: CCA-1111 CE, China) at speed of 45 rpm with water-bath temperature of 40°C and evaporation time of 30±10 minutes to evaporate the solvent. The dry extract was then collected from the flask and weighed using a digital balance (Mettler Toledo, Model: B303-S, Switzerland) and placed into an air tight glass vial before being stored at –20°C for further investigations.

Preparation of chemicals. The analytically graded chemicals and reagents were used in this research. A total of 3.6 ml Folic-Ciocalteu reagent was mixed in 32.4 ml of distilled water to attain 36 ml of 10-fold solution. Sodium carbonate at a concentration of 7.5% was prepared by melting 2.7 g of sodium carbonate (Na2CO3, purity > 99.5%) in 36 ml of distilled water using a magnetic stirrer (FAVORIT, Model: HS0707V2, Serial No: 7306) for 5 minutes. Stock solution of gallic acid was prepared by melting 10 mg gallic acid (C7H6O5.H2O, purity > 99.5%) in 10 ml absolute methanol.

For the analysis of total flavonoid compounds (TFC), 28.8 g of sodium nitrite ($NaNO_2$, purity > 98%) was placed into 144 ml distilled water and melted using magnetic stirrer for 5 minutes. Aluminum chloride at a ratio of 1:10 (w/v) solution was prepared by melting 1.08 g of aluminum chloride ($AlCl_3.6H_2O$, purity > 97%) in 10.8 ml of distilled water using a stirrer for 5 minutes. Also, 72 ml of 1-Mole sodium hydroxide solution was prepared by melting 2.88 g of sodium hydroxide (NaOH, M.W = 40.00 purity > 99%) in 72 ml of distilled water with a magnetic stirrer for 5 minutes. Quercetin stock solution was obtained by melting 5 mg of quercetin (Chem Faces, Cat. No: CFN99272, purity > 98%) in 5 ml of absolute methanol.

In order to evaluate antioxidant activities of the extract solutions, diphenyl-1-picrylhydrayl or DPPH, at 0.1 milli mole in a volume of 36 ml was prepared by melting (1.404 mg DPPH) in 36 ml of absolute methanol using a stirrer for 10 minutes.

Determination of extraction yield. The extraction yield (EY) was measured after the evaporation of solvent from the sample. The dried extract was collected from the rotary flask and weighed using digital balance (Mettler Toledo, Model: B303-S, Switzerland). The same procedures were repeated for all samples. Extraction yield was calculated according to the method developed by Izadiyan and Hemmateenejad (2016) using equation (1), and expressed in percentage.

$$\text{Extraction yield (\%)} = \frac{W_1}{W_2} \times 100 \qquad (1)$$

Where:
W1: is weight of extract after solvent evaporation (g)
W2: is weight of the leaf powder before extraction (g)

Determination of total phenolic content. The determination of total phenolic content (TPC) was carried out according to Ghasemzadeh et al. (2016) with minor modifications. A total of 2 mg dried extract was melted into 1 ml of absolute methanol and mixed well using an orbital shaker (Lab dancer, Model: 3365000, IKA) at a speed of 3,000 rpm for 1 minute. Then, from the solution 200 µL was taken and put in a test tube for analysis. After 1-minute, 1 ml of Folin-Ciocalteu reagent (10 fold) was added in the test tube and mixed well using the orbital shaker for 1-minute followed by the incubation for 10 minutes at total darkness in 25°C. The mixture was then added with 1 ml sodium carbonate (7.5%) and mixed using the orbital shaker at a speed of 3,000 rpm for 1 minute followed by incubation period for 30 minutes at total darkness at a temperature of 25°C. After incubation, the mixture was distributed in micro plate in triplicate and the absorbance was read at 765 nm using a spectrophotometer (Thermo Scientific, Model: 1510, Serial No: 1510-02520C, Fisher Scientific, Malaysia).

Eight different concentrations of gallic acid including 0, 25, 50, 75, 100, 125, 150 and 175 µg/ml were subjected to spectrophotometer at 765 nm for establishing calibration equation. Finally, the content of total phenolic of the sample was calculated according to the method of Genwali et al. (2013), using equation (2) and expressed as milligram of gallic acid equivalent per gram of dry extract (mg of GAE/g of DE).

$$TPC=C*V/M \tag{2}$$

Where:
C: is the concentration of gallic acid from calibration equation (mg/ml)
V: is the volume of tested sample (ml)
M: is the mass of the tested sample (g)

Determination of total phenolic yield. The total phenolic yield (TPY) was calculated to determine the precise amount of total phenolic compounds extracted from the samples. Considering that total phenolic yield could be affected by the extraction yield; TPY was calculated using the equation (3) and stated as TPY in milligram of gallic acid equivalent per 100 grams of dry weight of sweet basil leaves or TPY mg of GAE/100 g of DW.

$$TPY = TPC*EY \tag{3}$$

Where:
TPY: is the total phenolic yield (mg of GAE/100 g of DW)
TPC: is the total phenolic content (mg of GAE/ g of DE)
EY: is the extraction yield (%)

Determination of total flavonoid content. The total amount of flavonoid content (TFC) was determined using the method described by Ghasemzadeh et al. (2016) with minor modification. Dry extract (2 mg) was mixed in 1 ml of absolute methanol and mixed well using the orbital shaker at a speed of 3,000 rpm for 1 minute. The mixture was then poured into a glass test tube and mixed with 4 ml of sodium nitrite solution (1:5, w/v) using the orbital shaker at a speed of 3,000 rpm for 1 minute. The solution was incubated in total darkness at a temperature of 25 °C for 6 minutes. After 6 minutes, a total of 0.3 ml of aluminum chloride solution (1:10, w/v) was added to the solution and mixed well using the orbital shaker at a speed of 3,000 rpm for 1 minute. To complete the reaction, the solution was incubated for another 6 minutes under the same conditions as before. After 6 minutes, 2 ml of 1 mole sodium hydroxide was added following 10 minutes' incubation at 25°C in total darkness. The sample was replicated for three times into a micro plate and placed in the spectrophotometer, where the absorbance was read at 510 nm. For establishing calibration equation, nine different quercetin concentrations including 0, 40, 80, 120, 160, 200, 240, 280 and 320 µg/ml were introduced to the spectrophotometer at 510 nm and the absorbance was recorded. Finally, the total flavonoid content was calculated by using the equation (4) and expressed as milligram of quercetin equivalent per gram of dry extract (mg of QUE/g of DE).

$$TFC = C*V/M \tag{4}$$

Where:
C: is the concentration of quercetin from calibration equation (mg/ml)
V: is the volume of sample (ml)
M: is the mass of the sample (g)

Determination of total flavonoid yield. To determine the exact amount of total flavonoid compounds obtained from the samples, total flavonoid yield (TFY) was calculated (Hanudin et al. 2012). Taking this into consideration, total flavonoid yield could be affected by the extraction yield. TFY was calculated using the equation (5) and expressed as total flavonoid yield in milligram of quercetin equivalent per 100 grams of dry weight of sweet basil leaves or TFY mg of QUE/100 g of DW.

$$TFY = TFC*EY \tag{5}$$

Where:
TFY: is the total flavonoid yield (mg of QUE/100 g of DW)
TFC: is the total flavonoid content (mg of QUE/ g of DE)
EY: is the extraction yield (%)

Determination of antioxidant activity. The antioxidant potential of leaf extract was performed using method of Aadesariya et al. (2017) with minor modifications. Dry extract (0.1 mg) was melted in 1 ml of absolute methanol and mixed well using the electrical orbital shaker at a speed of 3,000 rpm for 1 minute. The mixture was then placed into a glass test tube. DPPH (0.1 mM, 1 ml) was added and mixed well using the electronic orbital shaker with speed of 3,000 rpm for 1-minute. The mixture was incubated for 30 minutes in darkness at a temperature of 25°C. At the same time, DPPH (0.1 mM, 1 ml) alone was used as a controlling variable. The absorbance of both sample and control was read at 517 nm wavelength using a spectrophotometer. Finally, antioxidant activity of the extract was calculated using the equation (6) and presented as percentage of inhibition.

$$\text{DPPH Inhibition \%} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100 \tag{6}$$

Where:
A control: absorbance value of control
A test: absorbance value of sample

RESULTS AND DISCUSSION

Extraction yield. The extraction yield was influenced considerably by the interaction of different temperatures and concentrations of aqueous ethanol solution. It was increased significantly, parallel with the increase in temperature and concentration of aqueous ethanol solution (Table 1). The lowest extraction yield was recorded from the combination of 60% aqueous ethanol solution + 40°C which was 5.94% only. Under 60% of aqueous ethanol solution, the extraction yield was increased to 8.08% and 10.34% as the temperature was increased to 60°C and 80°C, respectively. As the concentration of aqueous ethanol solution was increased to 75%, the same pattern was observed as in 60% aqueous ethanol solution, where there was an increased in extraction yield together with the increased of temperature. The extraction yield was increased from 6.41% to 10.39% as the temperature was increased from 40°C to 80°C under 75% aqueous ethanol solution. The maximum extraction yield was obtained from the use of the highest concentration of aqueous ethanol solution (90%) at 80°C.

Table 1. Effect of temperature and concentration of aqueous ethanol solution on extraction yield.

	Extraction yield (%) Aqueous ethanol (%, v/v)			
Temperature (℃)	**60%**	**75%**	**90%**	**Means**
40	5.94^{g}	6.41^{f}	7.42^{e}	6.59^{C}
60	8.08^{d}	9.03^{c}	9.17^{c}	8.76^{B}
80	10.34^{b}	10.39^{b}	11.56^{a}	10.77^{A}
Means	8.12^{C}	8.61^{B}	9.38^{A}	

In the same column and row, means showed with the same letters are not significantly different at $p < 0.05$ by Fisher's Protected Least Significant Difference test. The capital letter represents the analysis for two-way ANOVA (3 X 3) and the small letter represents the one-way ANOVA analysis.

Based on the results, both factors interacted with each other and positively influenced the extraction yield from dried leaves of sweet basil. Temperature is an important factor in enhancing soluble activities of plant materials during extraction (Efthymiopoulos et al. 2018; Silva et al. 2007; Spigno and De-Faveri 2007) as proven in this study. The mechanism behind stimulation of extraction process using varying temperatures can be attributed to several factors. The adjustment of temperature during the extraction is used to weaken the cell wall, making it easier for the solvent to enter the cell and extract the phytochemical constituents (Esclapez et al. 2011; Kushwaha et al. 2018). Dahmoune et al. (2015) The mechanism in which temperature stimulates the extraction process of phytochemicals is by decreasing the viscosity of solvent molecules (Dahmoune et al. 2015). A previous report revealed that temperature has the potential to destroy cells of plant material by increasing vapour pressure inside the cells, which leads to improved extraction (Zhang et al. (2008). Although high temperature is needed to extract the phytochemicals, it is however dependent on the type and concentration of solvent used. A low amount of extract will be obtained if the concentration of aqueous ethanol used is below 71% at temperature above 74°C in *Ilex kudingcha* (Sun et al. 2011).

Apart from this, aqueous ethanol solution at high concentration could enter the cells and leach the contents. The mechanism behind stimulation of extraction yield by using aqueous ethanol solution could be related to the ability of aqueous ethanol solution in making plant cells larger in order to increase contact surface between solvent and solute sample (Dahmoune et al. 2015). From this present study, the increase in the concentration of aqueous ethanol solution showed positive influence in solubility of phytochemicals in the form of solid particles of sweet basil dry leaves. The result of extraction yield in this study is consistent with an earlier study, where high percent extraction yield was obtained by combining high temperature with high concentration of solvent in Iranian sweet basil accessions (Izadiyan et al. 2016).

Total phenolic content. Data on the effect of different temperatures and concentrations of aqueous ethanol solution on TPC is presented in (Table 2). It was found that the interaction of temperature and aqueous ethanol's concentration had a significant influence on TPC. Extraction done at temperature of 40°C showed the lowest amount of TPC extracted from sweet basil leaves, particularly when extracted with 60% aqueous ethanol solution which was only 28.59 mg of GAE/g of DE. No significant increase was detected when the concentration of aqueous ethanol solution was increased to 75% under the same temperature. By increasing the concentration of aqueous ethanol solution to 90%, the amount of TPC extracted increased sharply at 48.90 mg of GAE/g of DE.

Under 60°C, increasing the aqueous ethanol solution concentration from 60% to 75% and 90%, resulted in a significant increase in TPC from 46.60 mg of GAE/g of DE to 49.52 mg of GAE/g of DE and 51.88 mg of GAE/g of DE, respectively. In contrast, under 80°C, TPC was significantly decreased from 67.02 mg of GAE/g of DE to 58.25 mg of GAE/g of DE and 55.18 mg of GAE/g of

DE by increasing aqueous ethanol solution concentration from 60% to 75% and 90%, respectively. The lowest and highest TPC were obtained through the combination of 40°C + 60% aqueous ethanol solution and 80°C + 60% aqueous ethanol solution which were 28.59 mg of GAE/g of DE and 67.02 mg of GAE/g of DE, respectively.

Table 2. Effect of temperature and concentration of aqueous ethanol solution on total phenolic content.

	Total Phenolic Content (mg of GAE/g of DE)			
	Aqueous ethanol (%, v/v)			
Temperature (°C)	**60**	**75**	**90**	**Means**
40	28.59^{g}	29.36^{g}	48.90^{e}	35.61^{C}
60	46.60^{f}	49.52^{e}	51.88^{d}	49.33^{B}
80	67.02^{a}	58.25^{b}	55.18^{c}	60.15^{A}
Means	47.40^{B}	45.71^{C}	51.99^{A}	

In the same column and row, means showed with the same letters are not significantly different at $p < 0.05$ by Fisher's Protected Least Significant Difference test. The capital letter represents the analysis for two-way ANOVA (3 X 3) and the small letter represents the one-way ANOVA analysis.

The results revealed that the maximum amount of phenolic compound was extracted at high temperature with low concentration of aqueous ethanol solution. The efficiency of solvent greatly depends on its polarity, where low concentration of aqueous ethanol solution to water (v/v) results in high polarity. According to Truong (2019), high polar compounds could be easily extracted by the high polar solvents. Since water is highly polar solvent (Zhang et al. 2007), lower concentration of aqueous ethanol solution provides solvent with high polarity potential. Since phenolic compounds are polar, they could be easily recovered by using polar solvent (Zhang et al. 2008). Therefore, it seems that most of the phenolics present in sweet basil leaves were highly polar compounds. This is in agreement with previous researchers who had confirmed the effective recovery of phenolic compounds from plant materials by applying low concentration of aqueous ethanol solution (Kalia et al. 2008; Mith et al. 2016; Ngo et al. 2017).

Total phenolic yield. Total phenolic yield (TPY) was investigated in order to find out which treatment was more sufficient in obtaining larger amounts of phenolic compounds from dry leaves of sweet basil. The results showed significant effect in TPY through the interaction of varying temperatures and aqueous ethanol solution concentrations (Table 3).

Table 3. Effect of temperature and concentration of aqueous ethanol solution on total phenolic yield.

	TPY mg of GAE/100 g of DW			
	Aqueous ethanol (%, v/v)			
Temperature (℃)	**60%**	**75%**	**90%**	**Means**
40	169.97^{g}	188.40^{g}	362.80^{f}	240.38^{C}
60	376.64^{f}	447.25^{f}	476.20^{d}	433.36^{B}
80	693.50^{a}	605.61^{c}	638.43^{b}	645.84^{A}
Means	413.36^{B}	413.75^{B}	492.47^{A}	

In the same column and row, means showed with the same letters are not significantly different at $p < 0.05$ by Fisher's Protected Least Significant Difference test. The capital letter represents the analysis for two-way ANOVA (3 X 3) and the small letter represents the one-way ANOVA analysis.

Under the temperatures of 40°C and 60°C, increasing the concentration of aqueous ethanol solution from 60% to 75% led to non-significant difference in TPY. By increasing the concentration of aqueous ethanol solution to 90%, under 40°C and 60°C, the extraction of TPY was significantly increased from 188.40 and 447.25 to 362.80 and 476.20 mg of GAE/100 g of DW, respectively. Under 80°C, increasing the aqueous ethanol concentration from 60% to 75% and 90% causes a significant decrease in TPY from 693.50 to 605.61, followed by an increase to 638.43 mg of GAE/100 g of DW, respectively.

In comparison to all temperatures used in this study, it was proven that higher temperature positively influenced the extraction of TPY from sweet basil leaves, whereas under all concentrations of 60, 75 and 90% aqueous ethanol solution, TPY significantly increased (Table 3). Overall, the lowest TPY was obtained from the combination of 40°C + 60% and 40°C + 75% aqueous ethanol solution with values of 169.97 and 188.40 mg of GAE/100 g of DW, respectively. The highest TPY was obtained through the combination of 80°C + 60% aqueous ethanol solution which is 693.50 mg of GAE/100 g of DW.

The higher extraction yield of phenolics at the highest temperature could be related to the solvent diffusive enhancement characteristic of temperature, where high temperature decreases the solvent surface tension, viscosity and stimulates diffusion of the solvent to activate it in order to extract phenolic compounds (Ilaiyaraja et al. 2015; Mohamad et al. 2010; Raj et al. 2020).

In this study, the extraction temperature at 80°C was enough to facilitate the recovery of phenolic compounds (Tables 2 and 3). This was in agreement with the extraction of phenolic constituents from citrus peel (Li et al. 2006).

Total flavonoid content. The extraction of total flavonoid content (TFC) in sweet basil leaves was affected by different temperatures and concentrations of aqueous ethanol solution with significant interaction. At 40°C, data on TFC showed a significant increase from 5.71 to 10.16 and 18.19 mg of QUE/g of DE, respectively, when the concentration of aqueous ethanol solution was gradually raised from 60 to 75, and 90% (Table 4). As the extraction was done at 60°C, the amount of TFC extracted was increased tremendously from 6.22 to 29.68 mg of QUE/g of DE at a concentration of 60 to 75% aqueous ethanol solution, respectively. However, there was no further increase of TFC when the aqueous ethanol concentration was at 90%.

Table 4. Effect of temperature and concentrations of aqueous ethanol solution on total flavonoid content.

	TFC mg of QUE/g of DE			
	Aqueous ethanol (%, v/v)			
Temperature (°C)	**60%**	**75%**	**90%**	**Means**
40	5.71^{f}	10.16^{e}	18.19^{d}	11.35^{C}
60	6.22^{f}	29.68^{c}	29.97^{c}	21.95^{B}
80	44.70^{a}	32.85^{b}	10.35^{e}	29.30^{A}
Means	18.87^{B}	24.23^{A}	19.50^{B}	

In the same column and row, means showed with the same letters are not significantly different at $p < 0.05$ by Fisher's Protected Least Significant Difference test. The capital letter represents the analysis for two-way ANOVA (3 X 3) and the small letter represents the one-way ANOVA analysis.

Similar to phenolics, a higher recovery of flavonoids was observed at combination of higher temperature with lower solvent concentration. This result agrees with previous findings where a maximum recovery of flavonoid compounds was reported at high extraction temperature (79.07°C and 94.66 °C) in *Euphorbia hirta* L. and *Flos populi* plants, respectively (Abu-Bakar et al. 2020; Sheng et al. 2013). The increase in extraction yield of flavonoid compounds could be attributed to a variety of temperature-related effects. It is well understood that higher temperature increased decomposition of dry leaf sample and facilitated the solubility of flavonoids into solvent (Ghitescu et al. 2015; Liu et al. 2010). On the other hand, lower concentration of ethanol to water (60% v/v) in this study was selected as potential solvent for extracting optimum flavonoids from sweet basil dry leaves. A similar trend was reported earlier in *Crinum asiaticum*, *Passiflora* spp and *Cyclocarya paliurus* (Yu et al. 2019; Gomes et al. 2017; Xie et al. 2015). It was also demonstrated that 80% aqueous ethanol solution blocks solubility of flavonoid compounds from plant cells (Yu et al. 2019). This could be due to the fact that increasing the concentration of ethanol in water would decrease solvent polarity. Therefore, this study suggests that the flavonoid compounds in sweet basil leaves are highly polar compounds such as rutin, kampferol, naringenin and quercetin (El-Nahal and Thabet 2012). The lower concentration of aqueous ethanol solution is therefore more sufficient in extracting these compounds compared to higher aqueous ethanol concentrations.

Total flavonoid yield. Total flavonoid yield (TFY) was calculated in order to differentiate whether the highest amount of total flavonoid is reached from the treatment which resulted in the highest dry extract or highest TFC. TFY increased by increasing the concentration of aqueous ethanol solution at low and moderate temperature while at higher temperature the opposite was observed. At 40°C, increasing the concentration of aqueous ethanol solution from 60 to 75 and 90% increased significantly TFY from 33.99 to 65.19 and 135.01 mg QUE/100 g DW, respectively (Table 5). Meanwhile, extraction done at 60°C showed greater strength to extract TFY where it reached 268.05 mg QUE/100 g DW in 75% of aqueous ethanol solution. Further increase in the aqueous ethanol solution concentration did not show a significant increment of TFY under 60°C. Furthermore, the highest TFY was recorded from 60% aqueous ethanol solution at 80°C which was 462.52 mg QUE/100 g DW.

Table 5. Effect of temperature and concentration of aqueous ethanol solution on total flavonoid yield.

	TFY mg of QUE/100 g of DW			
	Aqueous ethanol (%, v/v)			
Temperature (℃)	**60%**	**75%**	**90%**	**Means**
40	33.99^{h}	65.19^{f}	135.01^{d}	78.07^{C}
60	49.95^{g}	268.05^{c}	275.08^{c}	197.69^{B}
80	462.52^{a}	341.52^{b}	119.79^{e}	307.94^{A}
Means	182.16^{B}	224.92^{A}	176.63^{B}	

In the same column and row, means showed with the same letters are not significantly different at $p < 0.05$ by Fisher's Protected Least Significant Difference test. The capital letter represents the analysis for two-way ANOVA (3 X 3) and the small letter represents the one-way ANOVA analysis.

Antioxidant activity. The interaction between temperature and aqueous ethanol solution concentration had a significant influence in the magnitude of extraction of antioxidants (Table 6). Increasing the concentration of aqueous ethanol solution from 60% to 75%, significantly increased the antioxidant activities from 20.71 to 24.55% at 40°C. However, additional increase in the concentration of aqueous ethanol solution to 90% caused in the opposite.

At 60°C, gradual increment in concentration of aqueous ethanol solution from 60 to 75 and 90% led to a significant increase in antioxidant activities from 21.67 to 30.03 and 33.54%, respectively. Interestingly, at higher temperature of 80°C, increasing the concentration of aqueous ethanol solution from 60 to 75 and 90% significantly decreased the antioxidant activity from 66.80 to 42.19 and 40.84%, respectively. Although it showed a different trend, however, the extraction was recorded to be highest under this temperature.

Table 6. Effect of temperature and concentration of aqueous ethanol solution on antioxidant activity.

	Inhibition% (100 µg/ml)			
	Aqueous ethanol (%, v/v)			
Temperature (°C)	**60%**	**75%**	**90%**	**Means**
40	20.71^{g}	24.55^{f}	25.52^{f}	23.59^{C}
60	21.67^{g}	30.03^{e}	33.54^{d}	28.41^{B}
80	66.80^{a}	42.19^{b}	40.84^{c}	49.94^{A}
Means	36.39^{A}	32.26^{C}	33.30^{B}	

In the same column and row, means showed with the same letters are not significantly different at $p < 0.05$ by Fisher's Protected Least Significant Difference test. The capital letter represents the analysis for two-way ANOVA (3 X 3) and the small letter represents the one-way ANOVA analysis.

Altogether, it can be stated that gradual increase of temperature led to an increase in AA, under low concentration of aqueous ethanol solution. Phenolics and flavonoids are considered as main groups of antioxidant compounds in plants (Aryal et al. 2019; Jung et al. 2011; Scapin et al. 2016). Since these two groups of compounds and AA are higher at the same treatment, it is assumed that they are responsible for antioxidant activity of leaf extracts in sweet basil. This is proved by the results of correlation analysis in this study.

Inside any living cells, Reactive Oxygen Species (ROS) are radical compounds that cause the death, damage and degradation of cells and tissues (Chen 2021). These radicals could be removed by having the antioxidant activity in the tissues or cells (Sharma and Tomar 2021). The antioxidant constituents donate the hydrogen in the electron form and act as the radical intermediates; therefore, they are considered as the main ROS scavengers (Jeong et al., 2004). As a result, including antioxidants in foods and medicines can protect cells from damage and reduce the harmful effects of several diseases. Therefore, the increment in antioxidant compounds and their activities could be a great contribution to the pharmaceutical and nutritive industries.

Pearson correlation analysis. All variables were positively correlated with each other (Table 7). In particular, total phenolic and flavonoid contents were positively correlated with the extraction yield at r=0.98 and r=0.57, respectively. Similarly, total yield of phenolic and flavonoid were correlated positively with total content of phenolic and flavonoid at r=0.85 and r=0.64, respectively. Both groups of phenolic and flavonoid compounds shown positive correlation with antioxidant activity of the extract. This is supported by the other researchers who previously revealed positive correlation between phenolic compounds and antioxidant activity in *Bergenia ciliata* (Genwali et al. 2013), *Lantana camara* (Mahdi-Pour et al. 2012) and *Lens culinaris* Medik (Giannakoula et al. 2012). Interestingly, the correlation between total flavonoid content and antioxidant activity was stronger (r = 0.96) when compared with correlation between total phenolic content and antioxidant activity (r = 0.78). This suggests that flavonoids present in sweet basil leaves are highly correlated to the expression of antioxidant activity compared to phenolic compounds**.**

Table 7. Pearson correlation of extraction yield, antioxidant compounds and antioxidant activity in response to different temperature and concentrations of aqueous ethanol solution on sweet basil leaf extraction.

Variables	EY	TPC	TPY	TFC	TFY	AA
EY	1					
TPC	0.98**	1				
TPY	0.78**	0.85**	1			
TFC	0.56*	0.65*	0.74*	1		
TFY	0.98**	0.99**	0.84**	0.64*	1	
AA	0.70*	0.78**	0.86**	0.96**	0.78**	1

Significantly and highly significant expressed by * and **, respectively.
EY: extraction yield, TPC: total phenolic content, TPY: total phenolic yield, TFC: total flavonoid content, TFY: total flavonoid yield and AA: antioxidant activity.

CONCLUSION

The combination of optimal temperature and aqueous ethanol solution are important in extracting the total phenolic and flavonoid contents from sweet basil leaves. Both factors must complement each other in order to obtain high extraction efficiency. In particular, the optimal extraction yield for dry extract was obtained by using 90% aqueous ethanol solution at 80°C for 90 minutes. In addition to this, all the tested variables in this study positively correlated with each other. It is recommended to use 60% aqueous ethanol solution at 80°C for 90 minutes to obtain optimum total phenolic and flavonoid contents with optimum antioxidant activities. Future research is needed to identify other phytochemicals in sweet basil leaves under extraction conditions of 80°C using 90% aqueous ethanol solution.

CONFLICT OF INTEREST

We declare that there is no conflict of interest existing related to this work.

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DIETARY RISK ASSESSMENT OF FARMER PRACTICE IN BITTER GOURD (*Momordica charantia* L.) DIPPED IN INSECTICIDE SOLUTION

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ABSTRACT

A field study was conducted to investigate the dipping and spraying of bitter gourd fruits with insecticide solution at the National Crop Protection Center, University of the Philippines Los Baňos in January 2019. Bitter gourd fruits were sprayed with recommended dose and double the recommended dose of commercial products containing chlorpyrifos, profenofos and cypermethrin. The field trial results revealed all pesticide residues in both applications, after the post-harvest interval of 10, 7, and 7 days, respectively did not exceed the CODEX or ASEAN Maximum Residue Limits (MRLs) of several fruiting vegetables. A pesticide residue survey of 34 bitter gourd market basket samples were analyzed for various insecticide residues. A variable range of detectable insecticide residues were detected with chlorpyrifos, cypermethrin, endosulfan and profenofos. The effects of home processing were evaluated and the results demonstrated that washing with mild detergent solution was consistently a good home preparation for reducing residues substantially on bitter gourd fruits. In addition, soaking in vinegar alone, soaking in vinegar and boiling, as well as grilling also contributed to reduction of residues. A combination of home processing steps should be able to address food safety concerns. The dietary risk offered by dipping bitter gourd in chlorpyrifos, cypermethrin and profenofos is very low and similar in magnitude with that of spraying. The following maximum residue levels are recommended in bitter gourd: 0.2 mg/kg for chlorpyrifos and cypermethrin and 0.5 mg/kg for profenofos. Additional pesticide residue trials in the field need to be conducted to support an ASEAN MRL recommendation.

Key words: risk assessment, food processing, dietary risk

INTRODUCTION

Food safety is a public health priority. Dietary risk assessment of pesticide residues is an important component of food safety programs to ascertain risk. Bitter gourd (*Momordica charantia* L.) is a major part of the Filipino diet. Consumption of bitter gourd is on an average of 5 g/day or 2 kg/year representing a consumption of 0.6% of total food intake (FNRI 2014). Insecticide use in bitter gourd is meant to manage fruit flies, aphids, cutworms, cucurbit beetles, and leaf folders. Due to the enormity of the pest complex, farmers increase their insecticide use. In an informal survey with bitter gourd farmers and municipal agricultural officers, it was learned that some farmers resort to dipping bitter gourd fruits in insecticide solution as dipping entails less insecticide use, and in instances when farmers did not have a knapsack sprayer, application could still be done.

The Maximum Residue Limit (MRL) is the maximum concentration of a pesticide residue (expressed as mg/kg), to be legally permitted on or in food commodities and animal feeds. MRLs are based on Good Agricultural Practice (GAP) data and foods derived from commodities that comply with the respective MRLs are intended to be toxicologically acceptable (FAO 2022). These limits for pesticide residues in food are established by Codex Alimentarius Commission based on a risk assessment and limits for safe intake set by an FAO/WHO international expert scientific group named Joint FAO/WHO Meeting on Pesticide Residues (JMPR).

Agricultural products, particularly foods, are listed as one of the 13 priority integration sectors in the ASEAN Economic Community Blueprint. Different laws and regulations of individual countries with different MRLs would pose barriers to trade of food products within the region. Therefore, member states have been striving to harmonize MRLs of pesticides in agricultural produce traded in the region. ASEAN also started to establish its own MRLs, generated from residue trials that are aligned with international standards. To date, a total of 880 ASEAN MRLs has been established for vegetables and fruits, involving a total of 71 pesticides (ASEAN 2021). Current ASEAN and Codex MRLs are available for several fruiting vegetables such as cucumber, eggplant and tomato but none for bitter gourd (*Charantia momordica* L.) (CAC 2023).

Cypermethrin residues in bitter gourd were detected in Mauritius and four (4) samples contained residues (0.20 mg/kg) exceeding the FAO MRL (0.07 mg/kg) (Ministry of Agro Industry and Food Safety 2015). Bitter gourd sprayed with profenofos and bifenthrin contained residues exceeding the MRL at 24 hours after spraying (Mirani et al. 2012). In India, residues of dicofol, ∑- endosulfan were detected in bitter gourd (Srivastava et al. 2011). Bitter gourd samples from India were contaminated with organochlorine, and pyrethroid insecticides (Kumari et al 2002). Residues of carbofuran, chlorpyrifos, malathion, fenvalerate and endosulfan have likewise been detected in bitter gourd samples from Pakistan (Latif et al. 2011).

Food processing is a requirement of the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) for evaluation of pesticides for MRL recommendations. Freshly applied residues are easier to remove than aged residues. The type of wash solution also affects the extent of residue removal and detergent has been found to increase the amount removed (Holland et al. 1994). The magnitude of residues in bitter gourd needs to be assessed due to the corrugated nature of the fruit surface which could retain higher amounts of pesticide residues. Therefore, reducing the concentration of residues before consumption would help in protecting consumer health.

The effects of household processing on pesticide residues showed a reduction of pesticide residues in various vegetables (Ahmed et al. 2011; Calumpang 2014; Chung 2018; Đorđević and Đurović-Pejčev 2016; Kaushik et al. 2009). Pesticide residues can be lowered significantly by simple household practices like washing, peeling, and cooking operations (Soliman 2001; Zohair 2001). In India, bitter gourd was contaminated with parathion and permethrin. Washing and cooking minimized the residues of nine pesticides to a range of 1.74 to 64.78 and 38.40 to 90.15 percent, respectively (Joshi et al. 2015). Traditional Pakistani food processes such as washing, salting, blanching, sun-drying, dehydrating, and frying also reduced residues (Mirani et al. 2012).

There is virtually no formal information on pesticide application through dipping of bitter gourd. Therefore, there is a compelling need to generate data about this practice to determine the risks it may impose on consumers. Furthermore, food processing measures should be assessed to determine how practices at the household level can reduce pesticide residues. The information on the dietary risks of pesticide dipping practice can aid in the setting of ASEAN MRL for bitter gourd. The study sought to survey insecticide residues in market basket bitter gourd samples, determine the magnitude of insecticide residues in bitter gourd dipped with insecticide solution, identify food preparation methods to reduce insecticide residues and assess the dietary risk of insecticide residues detected.

MATERIALS AND METHODS

Method recovery test of bitter gourd samples. Bitter gourd fruits were quartered, and two opposite sides of the divided fruit were used as samples. The remaining two opposite sides were discarded. Samples were sliced thinly and blended until the consistency is a puree. Fifty-gram (50) samples were weighed, extracted and subjected to GC analysis. The method was optimised by recovery studies before the determination of pesticides in samples. Trials were conducted for bitter gourd using the Pesticide Monitoring Development Program (PMDP) multi residue method (Migano 1999) to determine acceptability of the method for analysis of six insecticides: chlorpyrifos, cypermethrin, endosulfan, fenitrothion, diazinon and profenofos, by gas chromatography. The PMDP method was modified slightly to extract bitter gourd with three replications spiked at 0.05 mg/kg. Average recoveries ranging from 80 to 120% were obtained. The same extraction procedure and gas chromatograph conditions as applied for recovery studies were used for sample analyses.

The gas chromatograph (Shimadzu 2010, Japan) was equipped with a Rtx-5ms capillary column and an electron capture detector (ECD). The injection volume for each sample was 1 µL with the injector and detector temperatures set at 280 and 320°C, respectively. The initial oven temperature was set at 120°C then increased to 250°C at the rate of 10°C/min and was held for 3 minutes, further increased to 270°C at 3°C/min and then raised to 310°C at 75°C/min and was held for 1.8 minutes. Confirmation of any residues detected was done using a gas chromatograph - mass spectrometer (GC-MS Shimadzu TQ8040) in the selective ion monitoring (SIM) mode.

Pesticide Residue Trials

Field preparation. Pesticide residues in bitter gourd fruit were evaluated in field trials by dipping and spraying fruit using the recommended rate of insecticide application. The study was conducted in January 2019. Proper irrigation and fertilization were maintained and pest management was observed.

Spraying field trials. Three rows of bitter gourd were sprayed, with a plastic sheet barrier around it to eliminate cross contamination. The insecticide was sprayed only once prior to sampling and fruits were sampled on selected days based on preharvest interval. Bitter gourd fruits were sprayed with chlorpyrifos, cypermethrin, and profenofos, using the recommended rate for either vegetables or watermelon. Priority insecticides were based on detections in market basket samples. About 1 kilogram of bitter gourd was collected at the same sampling schedule as that of the dipping trials.

Fig. 1. Dipping of bitter gourd fruit in insecticide solution in a plastic container.

Dipping trials. Bitter gourd fruits were dipped individually in the field. Each fruit was covered with a net bag and only fruits of marketable size were sampled. Four fruits (total of about 1 kg) were collected after dipping. Bitter gourd fruits were dipped in chlorpyrifos, cypermethrin, and profenofos, using both the recommended rate and double recommended rate.

Home preparation. Bitter gourd fruits were dipped in recommended rate and double the recommended rate solution. The fruits were harvested one day after spraying or dipping. All fruits were quartered longitudinally, and two opposite quarters were processed. Laboratory samples were sliced using a kitchen knife, blended (Osterizer 4172- 051, Oster, USA), extracted using acetonitrile, cleaned up using SPE C18 and Envicarb, and analyzed by GC-ECD. The following home processes were evaluated: unwashed, boiled in water for 15 minutes, unwashed, grilled for 20 minutes over electric stove top, washed with liquid detergent solution (0.05%), washed with liquid detergent and grilled or boiled, soaked with rubbing using a rubber foam and rinsed in tap water, soaked in mild vinegar solution (1%) for 5 minutes with mild rubbing using kitchen foam, soaked in vinegar and boiled for 15 minutes, and squeezed with salt, as in a salad and rinsed with water 3 times.

Dietary risk of insecticide residues detected. An estimated daily intake (EDI) was calculated (mg/kg body weight) for the insecticide evaluated (WHO 1997). The values were compared with the Acceptable Daily Intake (ADI) of the particular insecticide.

$$\text{EDI} = \frac{\text{Residue detected (mg/kg)} \ * \ 0.125 \text{ kg bitter gourd consumed per day}}{55 \text{ kg/body weight}}$$

The calculations used the following assumptions:

1. Daily intake of 0.125 kg of bitter gourd or ½ of medium sized fruit, representing a maximum daily intake.
2. Average weight of 55 kg body weight for Filipinos

RESULTS AND DISCUSSION

Field trials of bitter gourd fruits sprayed and dipped in insecticide solution.

Chlorpyrifos residues. Bitter gourd fruits were dipped or sprayed using the recommended rate. It appears that retention of chlorpyrifos residues in bitter gourd is not affected by the method of application, either spraying or dipping. Residues did not differ at 1 day after spraying or dipping and likewise at 10 days after application, which is the recommended pre-harvest interval (PHI) for chlorpyrifos in bitter gourd (Table 1). It is noted, that these residues, ranging from 0.001 to 0.005 do not exceed the CODEX or ASEAN MRLs of several fruiting vegetables. Consumption of 0.0125 kg bitter gourd per day would result in a consumption of 1.2×10^{-5} mg/kg bw chlorpyrifos per day, that corresponds to 0.12% and 0.10% of the ADI for dipped and sprayed bitter gourd, respectively at 1 day after application.

Table 1. Insecticide residues (mg/kg) in bitter gourd using the recommended rate.

Insecticide	Dipped	Concentration	Sprayed	Concentration
Chlorpyrifos	1 DAS	0.005	1 DAS	0.004
Chlorpyrifos	10 DAS *	0.001	10 DAS	0.001
Cypermethrin	7 DAS *	0.01	7 DAS	0.02
Profenofos	7 DAS *	0.024	7 DAS	0.018

*PHI based on label information, field conditions

A maximum residue level of 0.5 mg/kg is recommended for chlorpyrifos in bitter gourd, based on the residue levels in both sprayed (according to GAP) and dipped (non-registered use) which are lower than 0.2 mg/kg. This is the same for the existing ASEAN MRL for tomato (Table 2).

The decrease in chlorpyrifos residues over a period of 10 days may be influenced by factors including volatilization and photodegradation. Higher temperature favors volatilization and photodegradation due to hydrolysis, which occurs when chlorpyrifos is exposed to sunlight (Akoto et al. 2016). Under UV light or sunlight, chlorpyrifos undergo hydrolysis which occurs readily in presence of water at about pH 6 and very readily above pH 8. This releases its metabolite, 3,5,6-trichloro-2-pyridinol, which undergo further decomposition (JMPR 1972). Hence, the reduction of chlorpyrifos residues in bitter gourd when exposed to field conditions.

Table 2. Maximum Residue Limits (mg/kg) of test insecticides set for various fruiting vegetables.

Insecticide	Commodity	ASEAN MRL*	Commodity	CODEX MRL**
Cypermethrin	Fruiting vegetables, cucurbits	0.07	Fruiting vegetables, cucurbits	0.07
	Eggplant	0.03	Eggplant	0.03
	Tomato	0.2	Tomato	0.2
			Pepper, sweet	0.1
Chlorpyrifos	Tomato	0.5	Tomato	0.5
	Pepper, sweet	2	Pepper, sweet	2
Profenofos	Tomato	10	Tomato	10

*ASEAN MRL 2021 (FAO 2022a) ** CODEX MRL (CAC 2023)

Cypermethrin residues. Levels detected in sprayed (0.01 mg/kg) and dipped (0.02 mg/kg) bitter gourd did not decline up to 7 days and are essentially the same for either sprayed (according to GAP) or dipped (non-GAP practice) (Table 1). When cypermethrin is applied, the parent compound is the major identified residue with very little absorbed or translocated. Metabolites result from ester hydrolysis and hydroxylation processes. Exposed residues are subject to isomerisation, presumably by a photolytic process (JMPR 1972). Cypermethrin residues of dipped and sprayed bitter gourd also did not exceed the ASEAN MRL of eggplant and cucumber (0.2 mg/kg), a cucurbit like bitter gourd. Consumption of 0.0125 kg bitter gourd per day would result in a consumption of 3.0×10^{-5} mg/kg-bw cypermethrin per day, that corresponds to 0.06% and 0.08% of the ADI for dipped and sprayed bitter gourd, respectively at 1 day after application.

A maximum residue level of 0.2 mg/kg is recommended for cypermethrin in bitter gourd, based on the residue levels in both sprayed (according to GAP) and dipped (non-registered use) which resulted in residues that were much lower than 0.2 mg/kg. This value is the same for the existing ASEAN MRL for cucumber (Table 2).

Profenofos residues. Profenofos residues in bitter gourd fruits that were dipped or sprayed using the recommended rate contained residues of 0.02 mg/kg at 7 days, which is the recommended PHI for profenofos in bitter gourd. The retention of profenofos residues was not affected by the method of application (Table 1). Profenofos residues in dipped (0.024 mg/kg) and sprayed (0.018 mg/kg) bitter gourd did not exceed the MRL for tomato (2 mg/kg) and sweet pepper (0.5 mg/kg), which are both fruiting vegetables (Table 2). Consumption of 0.0125 kg bitter gourd per day would result in a consumption of 1.48×10^{-4} mg/kg-bw profenofos per day, that corresponds to 1.48% of the ADI. Profenofos is slowly absorbed and metabolized in plants. Profenofos was the major residue when harvested several weeks after the last application, and profenofos underwent hydrolysis of phosphate ester to form CGA 55960 4-bromo-2-chlorophenol and its sugar conjugate (JMPR 2008).

A maximum residue level of 0.5 mg/kg is recommended for profenofos in bitter gourd, based on the residue levels in both sprayed (according to GAP) and dipped (non-registered use) which resulted in residues that were much lower than the ASEAN MRL for sweet pepper (Table 2). Both vegetables (bitter gourd and sweet pepper) belong to the same food group of fruiting vegetables.

Survey of market basket bitter gourd samples. In some market basket samples, bitter gourd fruits contained detectable insecticide residues of the 4 commonly used insecticides fenitrothion, profenofos, chlorpyrifos, and cypermethrin. More detections were noted in the later part of 2018, and this coincided with the rise in market price of bitter gourd. It can be surmised that farmers used insecticides during this time to protect their harvest, thus terminal residues could be detected. A total of 34 samples were analyzed for various insecticide residues. Low level insecticide residues (less than 1 ppm) were detected. A total of 10 samples (30%) did not contain detectable residues, and 24 samples (70%) had detectable insecticide residues of chlorpyrifos, profenofos, cypermethrin and endosulfan. Trace levels of diazinon and fenitrothion were detected in only 2 samples. The most common insecticide detected was chlorpyrifos, in 50% of samples. Low levels of endosulfan, cypermethrin, profenofos, diazinon and fenitrothion were also detected. Some samples contained 2 or 3 insecticide residues but usually 1 insecticide was quantifiable with other(s) present in only trace amounts. The highest level detected was endosulfan (1.23 mg/kg), which is unauthorized use as it is no longer registered with the Fertilizer and Pesticide Authority since 2015 (Table 3).

The residue levels detected are below the recommended maximum residue level, except for the highest level detected in cypermethrin (Table 2). This information can be used by agricultural technicians to conduct farmer information campaigns in areas where use of insecticides may exceed the recommended dosage and/or timing. Additional information on pesticide management as well as information on biological control, intercropping and other pest management strategies are necessary so that farmer reliance on synthetic pesticides are minimized.

Table 3. Monitoring of insecticide residues in market basket bitter gourd samples. (n =34)

	Number of samples	**Residues detected (mg/kg)**	**Percent of samples analyzed**
Chlorpyrifos	17	0.001 – 0.084	50
Cypermethrin	5	Trace – 0.49	15
Endosulfan	3	0.001 – 1.23	9
Profenofos	1	Trace – 0.002	3
Diazinon	1	Trace	3
Fenitrothion	1	Trace	3

Home preparation of bitter gourd dipped at recommended rate. Home preparation is a good means to reduce insecticide residues. Boiling or cooking of unwashed bitter gourd dipped at the recommended rate, resulted in 85 to 90% reduction of residues of the 3 insecticides evaluated. Washing with mild liquid detergent, squeezing in salt and soaking in vinegar (as in a salad) and soaking in vinegar prior to boiling reduces chlorpyrifos, profenofos and cypermethrin residues considerably (Table 4).

Table 4. Percent reduction of selected insecticide residues using various home preparation methods.

Insecticide	**Washed, mild liquid detergent**	**Soaked in vinegar and boiled**	**Squeezed in salt and soaked in vinegar**	**Unwashed, Boiled**
Chlorpyrifos	78.4	71.6	62.0	89.0
Profenofos	63.5	61.4	63.5	90.0
Cypermethrin	55.2	64.0	38.1	84.6

Washing the bitter gourd dipped in either chlorpyrifos, profenofos or cypermethrin with mild liquid detergent solution reduced residues by 78.4%, 63.5 % and 55.2%, respectively. This is within the range reported in earlier studies for sweet pepper (62.58%) and eggplant (100%) (Radwan 2005). A 50-60% reduction in cypermethrin residues was determined in eggplant dipped in cypermethrin and subsequently washed with liquid detergent solution (Holland et al. 1994).

Soaking in mild vinegar solution and cooking bitter gourd in water resulted in significant reduction of insecticide residues in bitter gourd fruits that had been dipped. Our results are consistent with the previous studies which showed considerable reduction of initial residues by 68.5%, and 100% on eggplant and sweet pepper (Chandra 2015; Kiwango 2018).

Squeezing in salt then soaked in vinegar was meant to simulate preparing bitter gourd salad. Both organophosphate insecticides, chlorpyrifos and profenofos, were reduced substantially (about 60%) by the process while more cypermethrin residues were retained in the waxy layer of the bitter gourd skin (38% reduction). Cypermethrin is a non-polar insecticide and is expected to adhere to the waxy surface. (JMPR 2008).

Direct boiling of unwashed bitter gourd can also lead to substantial reduction of insecticide residues (85-90%). A reduction of 50-100% of several organophospate insecticides, such as chlorpyrifos, was observed for boiling (Satpathy et al. 2012). Hydrolysis of chlorpyrifos is known to be affected significantly by increased temperature (Hui et al. 2010). Boiling of vegetables was found to be more effective than washing in dislodging the residues in various fruiting vegetables. However, some intact residues or more toxic metabolites may be found in the boiled water (Chung 2018).

Alpha-cypermethrin and cypermethrin residues were found to be stable during hydrolysis conditions simulating pasteurization and boiling (JMPR 2011). The application of heat in cooking/boiling reduces residue levels and can enhance volatilization and hydrolysis (Holland et al. 1994). Cooking/boiling is the most effective treatment for reducing the residues of synthetic pyrethroids in different vegetables (Chauhan et al. 2014). Cypermethrin residues were reduced in bottle gourd and ridge gourd after cooking in water by 15–33% (Kadian et al. 2001). The age of the residues is an important factor in the retention of residues as these can move into the cuticular layer which is difficult to extract. This could explain the lower percent reduction of cypermethrin (15-33%) than this present study which used bitter gourd fruits that were collected one day after application.

Dipped at double recommended rate. These studies were conducted to assess a worst-case scenario when a double the recommended rate is used due to intense pest pressure in the field. Residues generated from a double recommended rate can likewise be reduced by washing with liquid detergent and subsequent boiling or grilling which resulted in substantial reduction of residues (44 to 90% reduction). These home processes are commonly practiced in Filipino households, like bitter gourd salad or sautéed mixed vegetables (*pinakbet*). Washing with mild detergent solution gave the highest reduction (69 to 80% reduction) that was consistent for all three insecticides tested. Soaking in vinegar as a preparatory step prior to grilling resulted in substantial reduction of initial residues (69 to 81%). Grilling by itself did not reduce much of the organophosphate insecticides, profenofos and chlorpyrifos, while 89% reduction was obtained by the pyrethroid, cypermethrin (Table 5).

Previous studies showed that the grilling process was most effective in reducing cypermethrin residues in eggplant (50%) while only 41% reduction was obtained by boiling in water (Walia et al. 2010). Roasting or grilling was found more effective than cooking in reducing insecticide residues in eggplant (Thanki 2012). In general, washing and soaking can only lead to a certain degree of reduction in residue level, while other processes such as peeling, soaking in chemical baths and blanching can reduce pesticide residues more effectively (Chung 2018).

Table 5. Percent reduction with home processes practice of the insecticide residues in bitter gourd dipped in double dose above the recommended rate.

Insecticide	Percent Reduction			
	Washed with liquid detergent, boiled	Washed with liquid detergent, grilled	Grilled	Soaked in vinegar and grilled
Chlorpyrifos	69.5	Not done	32.46	68.9
Profenofos	80.0	65.2	43.8	81.1
Cypermethrin	70.1	44.1	89.8	Not done

CONCLUSION

Seventy percent (70%) of the market basket bitter gourd samples bought from Metro Manila and Region 4A, Philippines wet markets and supermarkets had low level insecticide residues. Low levels of chlorpyrifos, endosulfan, cypermethrin and profenofos were detected in some samples. Home preparation methods can further protect consumers from exposure to insecticides residue. The greatest reduction in residues for the three commonly used insecticides were obtained by: boiling, washing with mild liquid detergent solution, soaking in vinegar and boiling, and soaking in vinegar and grilling. A combination of home processing steps should be able to address food safety concerns.

The dietary risk offered by dipping bitter gourd in chlorpyrifos, cypermethrin and profenofos is very low and similar in magnitude with that of spraying. All estimated daily intake values are below 1% of the ADI. The following maximum residue levels are recommended in bitter gourd: 0.2 mg/kg for chlorpyrifos and cypermethrin, and 0.5 mg/kg for profenofos. Additional pesticide residue trials in the field need to be conducted to support an ASEAN MRL recommendation. These may be conducted in the various countries in the ASEAN region.

The information gap on reduction of pesticide residues in specific food processes peculiar to traditional or modern Asian cuisine needs to be addressed especially in commodities that receive more pesticide applications due to the presence of a pest complex in the field and the increasing temperature under field conditions which hastens pest development. Food processing is the final step to safeguard consumer safety from dietary risk.

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PLASMA ANTI-MÜLLERIAN HORMONE LEVEL AND ITS RELATIONSHIP TO OVARIAN RESERVE, SUPEROVULATION RESPONSE, AND EMBRYO SURVIVAL IN BRAHMAN (*Bos indicus*) FEMALES

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ABSTRACT

Indicators of superovulation response is needed for efficient in vivo embryo production and facilitate production of genetically superior animals. Relationships of plasma Anti-Mullerian Hormone (AMH) levels to ovarian reserve, superovulation response, and embryo survival in Brahman females were examined on January 2019 in Manabí, Ecuador. Twenty-one Brahman females were classified according to plasma AMH levels arranged in a completely randomized design with factorial treatment. Factor A corresponded to animal classification (cows and heifers), and factor B corresponded to AMH levels (100<200, 200<300, and 300<450 ρg/mL). Animals were treated with progesterone implant, estrogen benzoate injection, and intramuscular progesterone on Day 0 and a decreasing concentration of gonadotrophin on Day 4-6. Prostaglandin injection and implant removal were done on Day 6. On Day 8, gonadorelin was given and artificial insemination performed. Ovarian reserve and superovulation response were assessed by endorectal ultrasonography. Embryos were collected, quality evaluated, transferred, and pregnancy determined. Positive correlations (R^2:0.73) between plasma AMH and number of ovarian reserves and number of transferable embryos were observed with increasing rates of 6.28% and 3.54%, respectively. Heifers had significantly higher corpora lutea than cows (13.7 *vs* 8.81, $P<0.05$). Embryo survival was highest in animals with higher AMH levels suggesting that selection of donors in superovulation programs should consider young animals with AMH levels above 300 ρg/mL for higher efficiency.

Key words: Follicle, corpora lutea, artificial insemination, MOET, embryos

INTRODUCTION

Anti-Müllerian hormone (AMH), also known as Müllerian Inhibiting Substance (MIS), is a homodimeric disulfide-linked glycoprotein that belongs to the transforming growth factor-β superfamily with a molecular weight of 140 kDa (Josso et al. 2001) corresponding to 553-575 amino acids and expressed only in gonads (Cate et al. 1986). It is expressed early in gonadal differentiation of the male before seminiferous tubules become morphologically distinct with the testis, causing the regression of Müllerian duct which in the normal female embryo develops into the reproductive tract

(Cate et al. 1986) thus, it is also called as MIS. In cycling females, it is produced by the granulosa cells of early antral follicles on the ovary (Monniaux et al. 2008; Rico et al. 2011) and is used as a marker for the ovarian reserve (Ireland et al. 2007 and 2008; Rico et al. 2009). The secretion of AMH is greatest in 2 to 5 mm follicles (Van Rooij et al. 2002) while the expression of AMH decreases as the follicles grow and enlarge, with expression essentially lost when the follicles reach 8 mm diameter or larger (Weenen et al. 2004). With the advances in reproductive biotechnologies concerning embryo production, understanding the role of AMH in the ovarian status of females is important, especially in superovulation treatment for *in vivo* embryo production. While effective *in vitro* embryo production results were achieved in cattle (Boni 2012; Morotti et al. 2014; Monteiro et al. 2017), improvement of the success rate of *in vivo* embryo production remains a challenge to decrease the cost of production and enhance efficiency and profitability. In *in vivo* embryo production, the goal of superovulation is to stimulate several small antral follicles to grow and mature, resulting in multiple ovulations. Therefore, the set of small antral follicles available for stimulation is of great importance in superovulation treatments.

Follicular counts and AMH levels in circulation represent valuable tools for predicting superovulation response due to its high correlation with ovulation and embryo recovery (Center et al. 2018). Similarly, cows with a large number of follicles had more recoverable and transferable embryos (Ireland et al. 2011).

Several studies on dairy cattle reported a strong positive correlation between circulating AMH levels and *in vivo* embryo production after superovulation, demonstrating that those animals with higher AMH levels had the best superovulation response that resulted in a greater number of corpora lutea, total embryo produced, and total transferable embryos (Monniaux et al. 2010; Rico et al. 2012; Souza et al. 2015; Aziz et al. 2017; Hirayama et al. 2017). The correlation was found in plasma AMH levels and ovarian follicle count after superstimulation treatment, the number of corpora lutea after superovulation (Rico et al. 2012; Rico et al. 2009; Souza et al. 2015), and the number of embryos produced in primiparous and multiparous cows (Souza et al. 2015). Circulating AMH levels were positively correlated with fertility by artificial insemination and natural service after the detection of spontaneous estrus in dairy cows (Ribeiro et al. 2014). Therefore, the evaluation of circulating AMH levels could help in increasing breeding efficiency and reproductive performance through the selection of animals for embryo production and insemination.

It should be noted that there are divergent results regarding the effects of AMH on superovulation response, which has been associated with the differential behavior of different cattle breeds. There is no relationship between AMH levels and *in vitro* embryo production rates in Holstein and Nelore females (Guerreiro et al. 2014), as well as young Nelore females (Zacarias et al. 2018). In the case of Japanese black cows, repeated superovulation sessions reduced the accuracy in predicting ovarian response by measuring AMH concentration because the ovarian response and plasma AMH concentration change progressively (Hirayama et al. 2017). These support the claim that superovulation can alter follicular development, oocyte maturation, ovulation, and sperm transport, which can ultimately affect normal fertilization and embryo development, resulting in a greater number of unfertilized oocytes and poor-quality embryos (Kafi and McGowan 1997).

Given the discrepancy in superovulation response in different bovine breeds, this study sought to evaluate the relationship between plasma AMH levels and ovulatory response in Brahman (*Bos indicus*) females.

MATERIALS AND METHODS

Location and animals. This study was carried out on January 2019 in the stable of the Aura Germania Company, located in the municipality of Paján, province of Manabí, Ecuador, at the coordinates LS 01°

33' 0" and LO 80° 25' 60", at an altitude of 110 masl, with an average annual temperature of 24º C, and average annual rainfall of 1500 mm which has the necessary facilities for animal handling, including embryo collection room and an insemination and embryo transfer chute, where animals receive the same management practices. The study utilized 21 Brahman females at the herd of the Aura Germania Company, selected based on the uniformity of age (1-4 yrs), parity (1), and body condition of 3-4 where 1 is emaciated and 5 is extremely fat. The animals were kept in paddocks, fed mainly on *Cynodon nlemfuensis* and *Panicum maximum* (Saboya) grass, and supplemented with concentrated minerals to meet nutritional requirements. Water is supplied *ad libitum* from natural sources. The analyses of the levels of plasma AMH were conducted in the Biotechnological Laboratory of the Polytechnical Agricultural College of Manabí Manuel Félix López (ESPAM-MFL), located in the municipality of Bolívar, province of Manabí, Ecuador at the coordinates, LS 0° 49' 23" and LO 80° 11' 01", and an altitude of 15 masl.

This study was carried out under the Animal Welfare Act Regulations of ESPAM MFL guidelines with approval granted by the Animal Ethics Committee of the National Institute of Agricultural Research of Ecuador with reference number ESPAM-MFL 830034001.

Experimental design. Cows and heifers selected based on the uniformity of age, parity, and body condition were subjected to blood sampling and subsequent determination of plasma anti-Müllerian hormone (AMH) levels in the blood. Three analysis groups were formed; the first group were animals with AMH levels ranging from 100<200 ρg/mL; the second group were animals with 200<300 ρg/mL AMH levels, and the third group were animals with 300<450 ρg/mL AMH levels. The relationship between AMH levels and the ovarian reserve which is the number and size of ovarian structures before and after superovulation treatment, the number of fertilized oocytes, and number of collected embryos after artificial insemination and *in vivo* collection, and the pregnancy rate after embryo transfer were examined.

Blood sample collection and hormone assay. Four mL of blood samples were collected three times at weekly interval before superovulation treatment from each animal. This was done by a puncture in the jugular vein using a gauge 16 x 1.5 disposable needle and blood was drawn by vacuum into a BD Vacutainer® NH tubes (Becton Dickinson and Co.) with sodium heparin. The blood samples were centrifuged immediately at 3,200 g for 10 minutes at 4 °C and instantly plasma was collected and stored at -20 °C until the ELISA test was performed.

For AMH determination, ELISA test, MOFA® "KITS" (AMH ELISA simple test Kit Bovine Serum, 40 samples Max / Kit 21700/100) was used for processing the samples. Plasma samples were thawed in a water bath at room temperature, briefly shaken on a "vortex" and centrifuged at 3,200 rpm for 10 minutes at 4 °C, then incubated for 12 hours at 4 °C in the presence of a first antibody, and for 1.5 hours at room temperature in the presence of a second antibody. A 50 µL of the sample was taken and AMH levels was read using the "Infinite 200 PRO Multimode Multiplate Reader" following the methods described by Rico et al. (2012).

Superovulation treatment. Heifers and cows were treated differently in terms of the concentration of Folltropin (Folltropin-V, Bionichi Animal Health Canada, Inc) and the schedule of the first dose of artificial insemination (AI). On Day 0, all donors received 0.5 g of controlled-release natural progesterone implant in a silicone device (DIB® 0.5, Zoetis, Madrid) and injected intramuscularly with 2.5 mg Estradiol Benzoate (Gonadiol, Zoetis, Madrid, Spain) and 50 mg P4 (Gestavec® 25, Vecol, Bogota, Colombia) at 6:00 h. On Day 4 to 7, Folltropin was administered in a decreasing manner with heifers given 30, 20, 20, 10 mg while the cows given 50, 40, 20, 10 mg in the morning and in the afternoon at 6:00 and 18:00 h, respectively. Each 20 mL vial of Folltropin contains FSH equivalent to 400 mg NIH-FSH-P1, diluted in a 20 mL vial of USP Bacteriostatic Sodium Chloride injection. On Day 6, PGF2 alpha (Cloprostenol base 250 ug/mL, Ciclase Dl, Zoetis, Madrid, Spain) was administered to all animals and the progesterone implant was removed. On Day 8, heifers were artificially inseminated at 6:00 h and 100 µg gonadorelin (Pig pituitary GnRH, Gonadorelin 0.005 g, Ceva Sante Animale, France) was injected at the 18:00 h while the cows were injected with 100 µg gonadorelin on 6:00 h followed by AI on the 18:00 h. On Day 9, heifers and cows were artificially inseminated at 6:00 h for the 2nd dose. On Day 15, the response to superovulation treatment was examined at 6:00 h.

Assessment of reproductive parameters and responses. To assess the superovulation responses of the treated animals, the number and size of follicles categorized as small (<4mm), medium (4-7mm), and large (>7mm) on their left and right ovaries were determined through endorectal ultrasonography attached with 7.5 MHz linear probe (Mindray Dp50 vet) at the time of artificial insemination. Additionally, the number of corpora lutea at the time of embryo collection before catheterization was determined by ultrasonography of the superovulated animals.

Embryo collection. Embryo collection was performed through transcervical intrauterine catheterization and uterine lavage using a Foley catheter 16 FG according to the methodology described by Vivanco (2002) and embryo quality was evaluated according to the IETS (Stringfellow and Seidel 2000) classification, which distinguishes the number of fertilized, degenerated and unfertilized embryos. The *in vivo* survival rates in transferred embryos were recorded to provide a reference on the pregnancy rate after embryo transfer of embryos derived from Brahman females with different levels of plasma AMH.

Recipient preparation. All collected embryos with transferable quality and appropriate stage (compact morula, blastula, expanded blastula, hatched blastula) were transferred fresh to recipient animals within the same stable on the 7th day from the beginning of the estrous cycle (Day 0-estrus), and on synchrony with the donor. The transfers were made by non-surgical procedures as described by Hufana-Duran et al. (2004).

Data analysis. The experiment consisted of a total of 21 animals. Descriptive statistics were used to analyze the variable number of embryonic structures such as the number of fertilized, degenerated and unfertilized oocytes; and the ovarian structures which are composed of the number of corpora lutea and anovulatory follicles; and the embryo recovery rates in Brahman females.

For the analysis of variance, a completely randomized experimental design with a factorial arrangement was used. Factor A corresponded to animal category (cows and heifers) and factor B corresponded to the AMH levels (100<200 ρg/mL, 200<300 ρg/mL and 300<450 ρg/mL) established from the blood samples. Each animal represented an experimental unit, and at least three repetitions were made for each treatment combination. The initial values of the quantified variables were previously examined through the Shapiro-Wilks test for normality and Bartlett's Test for Homogeneity of Variances. In the cases where the ANOVA assumptions were not met, the data were transformed for verification. The means were compared through the Tukey test at a 5% probability.

The degree of functional relationship among the quantified variables was determined through correlation and regression analyses. In the regressions, the number of ovarian structures was adjusted with the AMH ranges and transferable embryos. In Pearson´s correlation analysis, for the variables on embryonic qualities, transferable embryos, degenerated embryos, unfertilized embryos, corpora lutea, anovulatory follicles, ovarian structures and embryo recovery, the significance was determined through the Student's t-test at 5 and 1% probability. The statistical analyses were performed with the InfoStat® software (Di Rienzo et al. 2017).

RESULTS AND DISCUSSION

A total of twelve (12) heifers and nine (9) cows of single parity were used in the study according to their AMH level in blood plasma. Table 1 presents the profile of the animals used in this study.

Table 1. Category and number of animals used in the study.

AMH Level, ρg/mL	Heifer, n	Cow, n	Total
100<200	1	4	5
200<300	6	3	9
300<450	5	2	7
Total	12	9	21

AMH concentration and follicle number before superovulation. Comparison of the mean number of ovarian follicles in Brahman heifers and cow before superovulation treatment is presented in Table 2. Between heifers and cows within the same AMH level, no statistical difference was observed. A difference was observed among animal category where heifers had higher reserve of small follicles than cows; 37.17±1.70 vs. 34.22±1.70 ($p<0.05$), respectively. On AMH level, cows and heifers with 300<450 ρg/mL AMH had a significantly ($p<0.05$) higher number of small and medium size follicles, 47.08±3.33 and 46.00±4.71 against 30.05±2.72 and 37.00±4.36 and 25.54±3.48 and 28.50±4.71 follicles in 200<300 and 100<200 ρg/mL AMH groups, respectively.

The ovarian structures of the Brahman females with different levels of plasma AMH is presented in (Fig. 1). The total number of follicles was significantly ($p<0.05$) higher (46.54) in 300<450 ρg/mL AMH group than in 200<300 (33.52) and 100<200 (27.0) ρg/mL AMH groups.

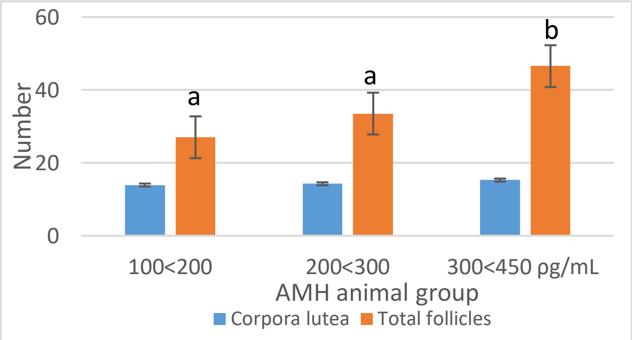

Fig. 1. Ovarian structures of Brahman females with different AMH plasma concentrations before superovulation treatment. Orange bars with different superscripts are different at $p<0.05$.

The higher number of ovarian follicles in Brahman females with >300 ρg/mL than those with <300 ρg/mL AMH plasma concentration indicates that the population of follicles contributes significantly to plasma AMH concentration. AMH is specifically expressed by ovarian granulosa cells in mammals (Vigier et al. 1984; Takahashi et al. 1986; Monniaux et al. 2008) making it a reliable endocrine marker of ovarian follicular population (Ireland et al. 2008; Rico et al. 2009, 2012; Monniaux et al. 2010; Souza et al. 2015). The number of small antral follicles is the direct target of ovarian stimulation treatments (Cardoso et al. 2018) and based on the results of this research, the plasma level of AMH is a good indicator in selecting Brahman females for superovulation treatment.

Table 2. Follicular reserve of Brahman heifers and cows before superovulation treatment.

Ovarian structures, M±SEM	Animal group according to AMH plasma concentration in ρg/mL							
	100<200		200<300		300<450		Total	
	Heifer	Cow	Heifer	Cow	Heifer	Cow	Heifer	Cow
Small follicle	16.33 ± 3.45^{a}	12.63 ± 2.55^{a}	22.85 ± 3.19^{abc}	17.72 ± 1.99^{ab}	30.0 ± 3.45^{c}	28.75 ± 2.44^{cb}	37.17 ± 1.70^{A}	34.22 ± 1.70^{B}
Medium follicle	12.16 ± 1.95^{a}	12.33 ± 1.13^{a}	14.14 ± 1.81^{a}	13.00 ± 1.44^{a}	16.00 ± 1.95^{a}	18.33 ± 1.38^{a}	14.10 ± 1.91^{A}	14.55 ± 1.31^{A}
Total follicles	28.50 ± 4.71^{a}	25.54 ± 3.48^{a}	37.00 ± 4.36^{abc}	30.05 ± 2.72^{ab}	46.00 ± 4.71^{bc}	47.08 ± 3.33^{c}	37.60 ± 4.59^{A}	34.22 ± 3.17^{A}

Means with different small letter superscript in same row are significantly different ($p<0.05$).
Means with different capital superscript (total heifer/cow) within row differs significantly ($p<0.05$).

Table 3. Analysis of variance for variables corpora lutea, anovulatory follicles, ovarian structures, transferable, degenerated, unfertilized oocytes, and embryo recovery rate in Brahman females.

Sources of variation	DF	Mean Squares						
		Corpora lutea†	Anovulatory follicles †	Ovarian structures †	Embryos†			Embryo recovery rates††
					Transferable	Degenerated	Unfertilized	
Animal categories	1	2.457*	1.099	0.558	0.492	0.159	3.524	29.304
AMH range	2	0.766	1.711	2.010**	4.417**	0.062	0.655	1160.026
Animal categories* AMH range	2	0.384	0.581	0.058	0.302	0.352	0.143	543.859
Error	15	0.571	0.732	0.312	0.192	1.020	1.258	691.857

† = Transformed data $(X+1)^{-0,5}$; †† = Transformed data arcsine(X); significant at $5^{(*)}$ and $1^{(**)}$ % probability for F test.

The higher follicular reserve in animals with >300 ρg/mL AMH plasma indicated that AMH concentrations have a high degree of relationship with the antral follicle count and these characteristics are good indicators in selecting animals for superovulation treatment as observed in Holstein cows (Benyei et al. 2003; Mossa et al. 2012).

AMH concentration and superovulation response. Analysis of variance for variables corpora lutea, anovulatory follicles, ovarian structures, embryos such as the transferable, degenerated, and unfertilized oocytes, and embryo recovery rate in Brahman females is presented in Table 3. Results showed a significant difference between animal categories i.e. heifer *vs.* cow, in the number of corpora lutea ($F=2.457$, $p<0.05$). In the AMH groups 100<200 ρg/mL, 200<300 ρg/mL and 300<450 ρg/mL, a significant difference was observed in the ovarian structures ($F=2.010$, $p<0.01$) and in the number of transferable embryos ($F=4.417$, $P<0.01$). No significant differences were found on the interaction between animal category and AMH range for the evaluated variables suggesting that in this study, the number of corpora lutea is the distinct indicator on the difference between heifer and cows and the ovarian structures and number of transferable embryos are the distinct indicators on the difference of the AMH level.

The number of superovulation response and quality of recovered embryos after superovulation treatment among AMH concentration groups are presented in Table 4. In these parameters, Brahman females in 300<450 ρg/mL AMH group had the significantly higher ($p<0.05$) number of ovarian structures i.e. anovulatory follicle and corpus luteum than the 100<200 AMH groups; 21.17 *vs* 10.67 ovarian structures. Transferable embryos were also highest in 300<450 ρg/mL AMH group than the other groups; 7.67 *vs*. 2.25 and 1.00 transferable embryos, respectively (Table 4). Similarly, a significant difference ($p<0.05$) was also observed on the embryo recovery rate between AMH groups though no difference was observed on the number of recovered degenerated and unfertilized oocytes. A significantly higher ($p<0.05$) number of corpora lutea was also observed after superovulation treatment in 300<450 AMH and 200<300 AMH groups (Fig. 2) suggesting that Brahman females with high AMH plasma concentration are best animals for superovulation that supports the studies conducted in other bovine species (Silva-Santos et al. 2014; Baruselli et al. 2015; Ghanem et al. 2016). Moreover, the number of the ovarian reserve is associated with the quality of oocytes (Ireland et al. 2009), embryonic competence (Tessaro et al. 2011), and fertility (Mossa et al. 2012; Ribeiro et al. 2014; Jimenez-Krassel et al. 2015).

Table 4. Superovulation response and quality of recovered embryos after superovulation of Brahman females with different class of AMH plasma concentration.

Parameters, Mean±SEM	**100<200**	**200<300**	**300>450 ρg/mL**
Ovarian structures*	10.67 ± 0.32^{a}	18.88 ± 0.27^{b}	21.17 ± 0.32^{b}
Transferable	1.00 ± 0.19^{a}	2.25 ± 0.16^{a}	7.67 ± 0.19^{b}
Degenerated	1.36 ± 0.43^{a}	1.35 ± 0.36^{a}	1.54 ± 0.43^{a}
Unfertilized	2.17 ± 0.48^{a}	2.11 ± 0.41^{a}	1.55 ± 0.48^{a}
Recovery rate, %	53.18 ± 11.39^{a}	61.01 ± 9.60^{ab}	81.53 ± 11.39^{b}

*Anovulatory follicles and corpus luteum.
Means with different superscripts within row differ significantly (Tukey $p<0.05$).

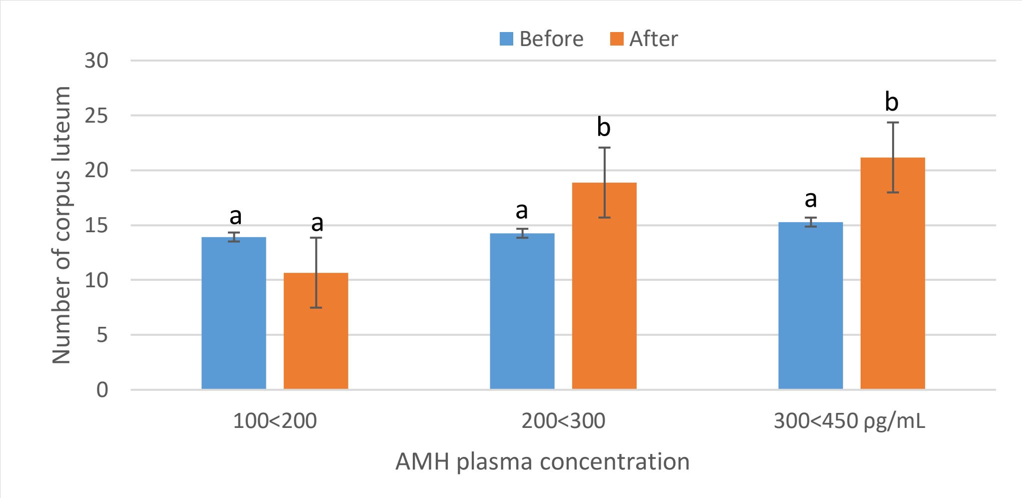

Fig. 2. Number of corpus luteum (CL) before and after superovulation treatment in Brahman females with different AMH plasma concentrations. Bars with different superscript letters within AMH plasma concentration are different at p<0.05.

The results after superovulation show that the high number of small follicular reserve have resulted in significantly higher number of follicles recruited to grow, mature, and ovulate resulting in the observed higher number of corpora lutea and embryos recovered in >300 ρg/mL AMH group. The number of follicles at the start of superovulation is positively correlated with the average number of corpora lutea and the number of collected embryos suggesting that the AMH level is a good indicator in the selection of Brahman females for superovulation. With the cost of superovulation treatment and the target of securing higher number of embryos for embryo transfer, selecting donor females with high efficiency is very important to optimize time and resources. With these results, it was deduced that examining the ovarian reserve and or checking the AMH level are important tools in the selection of donor animals in a multiple ovulation and embryo transfer program. These findings were also observed in beef cattle (Santos et al. 2016; Center et al. 2018) and in Nelore and Holstein breeds (Guerreiro et al. 2014). Though a high variation depending on the type of animal and high repeatability within individuals exist (Morotti et al. 2017), the observations that correlation of plasma AMH concentration on the number of collected and transferable embryos was weaker during the gestation period than during the periods prior to artificial and postpartum insemination in Japanese black cattle (Nabenishi et al. 2017); the concept that an antral follicle count is a useful tool for embryo production is reinforced by the impact on embryo production for genetic gain and reproductive performance. The plasma AMH concentrations before superovulation vary among animals but this correlates positively with the number of ovulations and transferable embryos produced which conforms with earlier reports in dairy cattle (Monniaux et al. 2010; Rico et al. 2009). With these, it can be deduced in this study that plasma level of AMH is a positive endocrine test in selecting Brahman females for superovulation treatment with checking the ovarian reserve as a physical indicator that a technician can perform in the absence of endocrine test and ultrasonography.

Animal category. The comparison of means by the Tukey test at 5% probability among the categories (Table 5) indicates that corpora lutea has the highest mean value (13.7±1.33) for heifers after superovulation, which were statistically higher (P<0.05) than those observed for cows (8.81±1.25). These results show that heifers had the higher number of <4 mm follicles than cows suggesting that the largest follicle population is present in young animals and gradually decreases as the animal ages. The decrease in follicular reserve starts when the animal reached puberty and undergo the regular estrous cycle where a follicle is recruited to grow, ovulated or reached terminal follicular growth and atresia (Monniaux et al. 2008; Rico et al. 2009; Macias-Andrade et al. 2020). The large follicular population in heifers resulted in the significantly higher corpora lutea observed after superovulation treatment than cows (Table 4) suggesting that heifers are potential targets for superovulation treatment.

Table 5. Number of corpora lutea after superovulation in Brahman heifers and cows.

Category	Mean±SEM
Heifers	13.70±1.33[a]
Cows	8.81±1.25[b]

Means with different superscript are statistically different (Tukey p≤ 0.05).

In both heifers and cows, higher numbers of small follicles were distinct in >300 ρg/mL AMH group. These findings show that AMH concentrations represent an excellent endocrine marker of the number of small antral follicles that constitute the direct target of ovarian stimulation treatments in Brahman. This was also observed in cows of Normande and Holstein breeds (Monniaux et al. 2010). These results demonstrate that the antral follicle count and AMH plasma concentration are determining characteristic in bovine females and it has great influence on the efficiency of reproductive biotechnologies and reproductive performance. These findings provide a good reference in selecting the donor for a multiple ovulation and embryo transfer program for livestock genetic improvement.

The quantitative cause-effect relationships of the AMH levels are shown in Fig. 3. For the number of ovarian structures, a direct linear relationship was observed, represented by the equation $\hat{y}=0.0628x-0.43$ with a $R^2=0.73$ (Fig. 3A); while for the number of good quality embryos, a direct relationship was also found, represented by the equation $\hat{y}=0.0354x-6.04$ with a $R^2=0.73$ (Fig. 3B). It verified that as AMH levels increase, the number of ovarian structures increases by 6.28% and the number of good quality embryos increases by 3.54%.

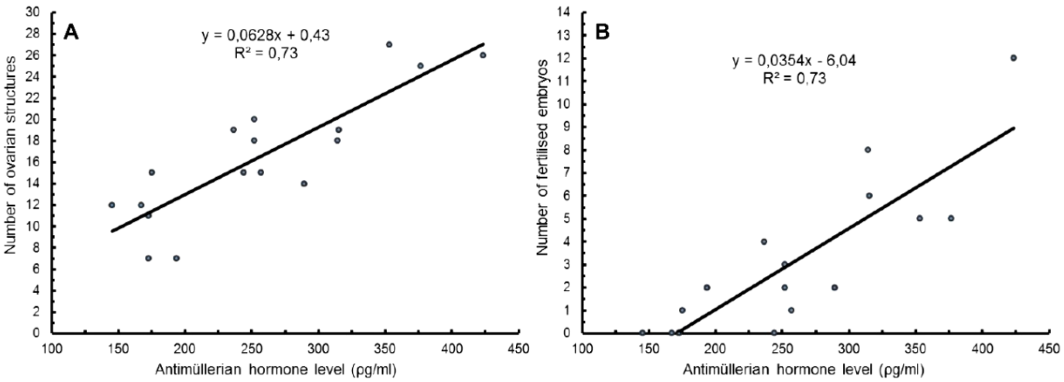

Fig. 3. The relationship among antimüllerian hormone levels, number of ovarian structures (A) and number of fertilized embryos (B) in Bos indicus bovine females.

Correlation coefficients of variables associated with superovulation response in *Bos indicus* Brahman heifers is presented in Table 6. Significant positive correlations were observed in embryonic structures and transferable embryos ($r=0.459$, $p<0.05$), degenerated embryos ($r=0.559$, $p<0.01$), unfertilized oocytes ($r=0.478$, $p<0.05$), corpora lutea ($r=0.882$, $p<0.01$), ovarian structures ($r=0.656$, $p<0.01$), and embryo recovery ($r=0.693$, $p<0.01$).

Table 6. Correlation coefficients of variables associated with superovulation response in *Bos indicus* heifers.

Variables	Embryonic structures	Transferable embryos	Degenerated embryos	Unfertilized oocytes	Corpora lutea	Anovulatory follicles	Ovarian structures	Embryo recovery
Embryonic structures	1	0.036	0.008	0.028	0.001	0.333	0.001	0.001
Transferable embryos	0.459*	1	0.438	0.267	0.087	0.911	0.128	0.034
Degenerated embryos	0.559**	0.179	1	0.252	0.021	0.761	0.060	0.211
Unfertilized oocytes	0.478*	-0.254	-0.262	1	0.051	0.377	0.290	0.159
Corpora lutea	0.882**	0.383	0.499*	0.431	1	0.091	0.002	0.088
Anovulatory follicles	-0.222	-0.026	-0.071	-0.203	-0.378	1	0.031	0.621
Ovarian structures	0.656**	0.343	0.417	0.242	0.639**	0.471*	1	0.036
Embryo recovery	0.693**	0.464*	0.285	0.319	0.381	0.115	0.459*	1

Correlation coefficient below the main diagonal. The probability associated with the test above the main diagonal.
Significant at 5(*) and 1(**) % probability for F test.

These results show that the number of embryonic structures and retrieval after superovulation depend on the ovarian structures present before superovulation treatment and this is associated with the number of corpora lutea present on the ovary. The quality of the retrieved embryos is classified as transferable, degenerated, and unfertilized and while all these parameters increased, the number of degenerated embryos showed higher correlation suggesting that as the number of embryonic structures increased after superovulation treatment, a high degree of degenerated embryos was also observed. The high incidence of degenerated embryos found in this study was not investigated but this could be due to aneuploidy (Hufana-Duran, 2009) as the embryos are of different stages of development. The number of degenerated embryos is also correlated with the number of corpora lutea ($r=499$, $p<0.05$) which means that these degenerated embryos are from ovulated oocytes fertilized by a sperm cell(s) that for unknown reason failed to develop to good quality and transferable embryos. The events involved in the embryo growth and survival are directly or indirectly related to cytokines, steroids, metabolites, and growth factors that when one of these compounds fails, it normally leads to the death of the embryo (Valadao et al., 2018). By triggering the ovaries to have more follicles to grow and develop during the superovulation treatment, limitations in these physiologic factors resulted in the high incidence of degenerated embryos and this is related to the high correlation of anovulatory follicles with ovarian structures ($r=0.471$, $p<0.05$). While superovulation treatment has resulted in higher ovarian structures; the mechanism for ovulation, normal oocyte maturation, and fertilization have to be taken into consideration to avoid anovulatory follicle and degenerating embryos. Considering these factors during the treatment might help improve the retrieval of transferable embryos.

The transferable embryos increased as embryo recovery increased ($r=464$, $p<0.05$) suggesting that the skill of the technician performing the *in vivo* collection of embryos is very important. The technician has to ensure that all the embryos present in the uterine horn are retrieved to increase the number of transferable embryos.

Embryo survival after embryo transfer. Out of the transferable embryos recovered from Brahman females, pregnancy and full-term development of calves were 0 (0/2), 50.0% (3/6), and 57.14% (4/7) in 100<200, 200<300, and >300 ρg/mL AMH groups, respectively (Table 7). While the number of transferred embryos were limited, these results show that the presence of AMH in the system is helpful in improving the development potential of the resultant embryo. It has to be noted that AMH is a key mediator in regulating steroidogenesis inhibiting estradiol secretion by reducing the expression of the aromatase enzyme CYP19 (Eilsø Nielsen et al. 2010) and in regulating progesterone production in granulosa cells *in vitro* (Yding Andersen et al. 2008). These observations suggest that the >300 ρg/mL AMH levels in the >300 AMH group is contributory in providing the developing embryos a conducive environment to grow and acquire the developmental competence needed resulting in higher pregnancy rate after embryo transfer.

Table 7. Pregnancy rate after transfer of embryos collected from the different AMH groups of Brahman heifers and cows.

Category	**Pregnancy rate (%) of AMH Group, ρg/mL**			
	100<200	**200<300**	**300<450**	**Total (Ave.)**
Heifer	0 (0/1)	66.67 (2/3)	50.00 (2/4)	50.00 (4/8)
Cow	0 (0/1)	33.33 (1/3)	66.67 (2/3)	42.86 (3/7)
Total/Ave.	0 (0/2)	50.0 (3/6)	57.14 (4/7)	46.67 (7/15)

The reduction of estradiol concentration and increase progesterone level during fertilization and early stage of development of the embryos in >300 AMH group are contributory to the higher developmental competence among embryos retrieved from donors in the >300 AMH group. This claim is reinforced by the observation in this study where a higher number of corpus luteum is present in >300 AM group and has a significant relationship with the number of fertilized and transferrable embryos. These results suggest that the AMH concentration in the blood is a potential reference in selecting females with good superovulation response in Brahman females.

The results of this study demonstrated that circulating AMH has significant positive correlations with follicle count, ovulation rate, the number of recovered embryos, and embryo survival in Brahman females. These findings are consistent with earlier reports in beef cattle (Center et al. 2018), in *Bos indicus* (Nelore), and *Bos taurus* (Holstein) donors (Zangirolamo et al. 2018). With these results, the circulating AMH level is recommended as a tool in the selection of Brahman females for superovulation treatment to ensure more efficient and profitable application of the MOET activities. It can be used as an auxiliary tool for selecting donor cows for embryo production in multiple ovulation and embryo transfer program and can also be applied for oocytes retrieval in ovum pick up activities.

The positive correlation among plasma AMH levels and ovarian follicle count and corpora lutea after superovulation were also observed in Holstein cows (Rico et al. 2012; Rico et al. 2009) and in the number of embryos produced in primiparous and multiparous cows (Monniaux et al. 2010; Souza et al. 2015). These results suggest that AMH can be used as a predictive physiological marker for fertility in Brahman females. This claim is supported by earlier reports in Holstein cows undergoing an ovulation synchronization protocol, since cows with high AMH levels (> 300 ρg / mL) had a higher pregnancy rate after artificial insemination, required less services per conception, and had fewer day open compared to cows with normal levels (<300 ρg / mL) (Aviles et al. 2017). While it has been determined that there are differences in serum levels of *Bos indicus* and *Bos taurus* heifers (Batista et al. 2014) and even within breeds of the same species (Ribeiro et al. 2014), the results of this study demonstrated that more embryos are recovered from Brahman females with higher AMH levels, and therefore with highest follicle count.

Embryo production rate in cattle after superovulation is difficult to predict and varies between individuals (Monniaux et al. 2010). The results suggest that the selection of donors of female *Bos indicus* embryos should consider relatively young animals with plasma AMH levels above 300 ρg/mL. The blood measurement of AMH is of great value in determining the potential of a donor cow in producing transferable embryos.

CONCLUSION

The AMH concentrations are endocrine markers in selecting animals for superovulation and of the number of small antral follicles that are gonadotropin-responsive. The female Brahman (*Bos indicus*) with a high concentration of AMH (>300 ρg/mL) has a significantly higher follicular reserve, which after superovulation treatment, resulted in a higher number of corpora lutea and retrieved transferable embryos. These embryos possess higher developmental competence demonstrating the highest potential of animals with high AMH levels be used in superovulation treatment for *in vivo* embryo production. Heifers have a significantly higher superovulation response than cows. It is reasonable to emphasize the appropriateness of selecting relatively young *Bos indicus* females with plasma AMH levels greater than 300 ρg/mL, or cows from the upper quartile of the evaluated herd.

According to the literature and data cited above, AMH seems to be correlated with several fertility parameters, and it may be a tool that can contribute to the success of embryo production both *in vivo* and *in vitro*. However, there is a great need to study the real long-term impact of AMH on fertility, to establish specific parameters of AMH classification, and to understand the physiological causes of the variation in the AMH among individual female cattle.

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FIRST REPORT OF *Telenomus remus* Nixon (Hymenoptera:Scelionidae) PARASITIZING *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) EGGS, ITS FIELD AND LABORATORY PARASITISM AND SOME BIOLOGICAL PARAMETERS IN THE PHILIPPINES

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ABSTRACT

Spodoptera frugiperda (J.E. Smith) is an invasive species that has recently emerged as a serious pest of corn in the Philippines. To address this issue, efforts are underway to develop biological control methods for managing this pest. This investigation led to the pioneering discovery of *Telenomus remus* Nixon, a potential parasitoid of *S. frugiperda* eggs, in cornfields located in Pangasinan, Laguna, and Quezon from January 2021 to June 2023. The findings revealed varying rates of parasitism by *T. remus* on *S. frugiperda*, ranging from 80% egg-mass parasitism from Tayabas, Quezon, 10% from Bocboc, San Carlos City in Pangasinan, and 45.7%for egg-masses from the Central Experiment station (CES), University of the Philippines Los Baños, College, Laguna. The progenies exhibited parasitism rates, under laboratory conditions, reaching as high as 87.5% for egg-masses and 83.1 % for eggs by *T. remus* collected from Barangay Bocboc, San Carlos City (Pangasinan). In the CES population, a 100% egg-mass and 90.3% eggs parasitism of FAW was recorded. A DNA barcode of 633 bp was successfully generated (GenBank Accession No. OR619425), providing valuable molecular identity information for future comparative studies. *T. remus* undergoes complete metamorphosis, with the development of various stages from egg to adult, and it typically takes 9-10 days for the Bocboc population and 11-12 days for the CES-UPLB population. These results highlight the potential of *T. remus* as a natural enemy capable of exerting effective control over *S. frugiperda* populations. This is the first report of *T. remus* parasitizing *S. frugiperda* eggs in the Philippines. The information gathered from this study contributes to the growing body of knowledge on biology and potential utilization of *T. remus* as a biocontrol agent against *S. frugiperda*. Further research is necessary to investigate on some aspects of its biology and mass rearing, its effectiveness in the field and practicality of deploying *T. remus* as a viable pest management strategy in corn cultivation in the Philippines.

Key words: biological control, egg parasitoid, fall armyworm, integrated pest management, parasitism

INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith), is a highly destructive pest with a wide range of host plants, including economically important crops like corn, rice, sorghum, and sugarcane (Montezano et al. 2018; Navasero et al. 2019). Originating from the Americas, it has become a significant threat in Africa (Georgen et al. 2016; Prassana et al. 2018) and Asia (Sharanabasapa et al. 2018). In recent years, the fall armyworm has been detected in the Philippines (Navasero et al. 2019; IPPC 2019) and has rapidly spread throughout the country.

The economic impact of this pest is staggering, with estimated crop losses reaching up to 13 billion US dollars annually in Africa (Day et al. 2017). In the Philippines, the government projected economic losses of around P20 billion in 8,000 to 12,000 hectares of conventional corn varieties from June to October 2020 (DA Communications Group 2020). The fall armyworm is predicted to affect corn production in the country, reducing it from 2.5 million metric tons to 1.6 million metric tons (Miraflor 2020). This infestation poses a significant threat to feed millers, food processors, livestock and poultry raisers, traders and consolidators, as well as consumers of corn and its by-products.

Currently, chemical control remains the primary strategy for managing the fall armyworm, although some experiments on entomopathogenic nematodes (Felicitas et al. 2022) and bacteria (Latina and Caoili 2023), entomopathogenic fungi, and viruses (Montecalvo and Navasero 2021 a &b) have been conducted in laboratory settings. However, there is a lack of research on parasitoids. In the Americas and the Caribbean Basin, approximately 172 species of parasitoids and parasites have been reported (Molina-Ochoa et al. 2003). Among these, the egg parasitoid *T. remus* shows promise as a potential biological control candidate (Kenis et al. 2019; Dong et al. 2021); it is aggressive and efficient ability to parasitize the large egg masses with multiple, superimposed layers covered with scales that limit the attack from other parasitoids; it can penetrate all the layers of the egg mass resulting in 80-100% parasitization in laboratory studies (reviewed by Hay-roe et al. 2015). *T. remus* has been observed to attack eggs of various *Spodoptera* spp. as well as other species belonging to the families Noctuidae, Pyralidae, and Arctiidae of the order Lepidoptera (Wojcik et al. 1976; Cave 2000; Tang et al. 2003). Parasitism of FAW by *T. remus* has been reported in Africa, India, and China (Li et al. 2019; Ning et al. 2019; Wang et al. 2020). However, *T. remus* is not included in the earlier listing of associated natural enemies of FAW in the Philippines (Navasero et al. 2019; Navasero and Navasero 2020; Valdez et al. 2023).

Telenomus remus is widely utilized in numerous countries as a biological control agent against *S. frugiperda* and currently being mass reared and released in several Central and South American countries (Cave 2000; Hay-roe et al. 2015). This egg parasitoid is naturally found in peninsular Malaysia and Papua New Guinea (Wengrat et al. 2021). It has been introduced into the Western Hemisphere in multiple locations and times with successful establishment in the Caribbean, Venezuela, and Honduras (reviewed by Cave 2000; Hay-roe et al. 2015) and with evidence for permanent populations in Ecuador (Hay-roe et al. 2015). It has been used as a biological control agent against various *Spodoptera* pest species. Previous studies have examined the biology and ecology of this parasitoid (reviewed by Cave 2000). *T. remus* is known for its high fecundity and its ability to effectively parasitize eggs of *Spodoptera* spp. even when they are located deep within an egg- mass. It also possesses strong dispersal capabilities and efficient host searching behavior, making it suitable for augmentative biological control programs. Notably, *T. remus* was released on a large scale (several thousands of hectares) in Venezuela during the 1990s as part of an integrated pest management (IPM) program targeting fall armyworm (FAW) in maize (Colmenarez et al. 2022). These releases resulted in a significant reduction in insecticide use against FAW, ranging from 49% to 80%. *T. remus* achieved impressive rates of FAW egg parasitism, reaching up to 90% following the releases (Colmenarez et al. 2022). Early reports in Brazil, indicated parasitism of *S. frugiperda* egg-masses by *T. remus* reaching 54-99% in maize, cotton, and soybean fields (Pomari et al. 2013). Other research studies have demonstrated the effectiveness of *T. remus* as a biological control agent against various lepidopteran pests, particularly in agricultural systems (Agboyi et al. 2021). Its use offers an environmentally friendly and sustainable approach to pest management, reducing the reliance on synthetic pesticides and promoting the conservation of natural enemies in agroecosystems.

Molecular identification plays a crucial role in accurately identifying species within the genus *Telenomus*, primarily due to the challenges posed by the close morphological similarity among species (Wengrat et al. 2021). This is particularly important in biological control programs, where the correct identification of biological control agents is essential for their success (Wengrat et al. 2021). DNA-based approaches have demonstrated their utility in characterizing closely related or cryptic species, thereby improving the accuracy of species identification in biological control work (Cengiz et al. 2016). Molecular identification techniques have been used to confirm the presence of *T. remus* in various regions (Agboyi et al. 2020; Wengrat et al. 2021). Identifying *T. remus* molecularly is critical for species resolution as a biological control agent.

In this paper, the first-time occurrence of *T. remus* in corn fields parasitizing eggs of *S. frugiperda* is reported in the Philippines. Morphological and molecular profiles and some biological parameters such as life stages, egg to larval periods and sex ratio of *T. remus* are presented.

MATERIALS AND METHODS

Field collection. Egg-masses of *S. frugiperda* were collected from corn fields in Luzon Island Philippines (Table 1). These were brought to the laboratory and individual egg-masses were placed in 10 cm diameter plastic plates and kept at 27°C, 50-60% RH and 12L:12D light cycle and checked daily for neonates of *S. frugiperda* or *T. remus* adult parasitoids. Emerging FAW larvae were promptly removed to prevent egg damage, transferred onto fresh leaves of 10-15 days after sowing (DAS) IPB var 6, reared to adulthood, and allowed to mate and produce eggs for parasitization. This was done for the population of *T. remus* collected from Barangay Bocboc, San Carlos City in Pangasinan and those from the Central Experiment Station, UPLB, Los Baños, Laguna. Those from Barangay Isabang, Tayabas, and Quezon were retrieved from preserved collections of FAW and associated organisms.

Table 1. Site of collection, date, host plant and number of egg-masses of *Spodoptera frugiperda* (J.E. Smith) with emerged adults of *Telenomus remus* Nixon parasitoids.

Locality	Collection Date	Host Plant	No. Egg Mass
Barangay Isabang, Tayabas, Quezon 13.96077 N 121.5584227 E	January 21, 2021	Corn	15
Barangay Bocboc, San Carlos City, Pangasinan 15.8934842 N 120.2664141 E	February 4, 2023	Corn	50
Central Experiment Station, Pili Drive, UPLB, College Laguna 14.09589 N 121.15117 E	June 5, 2023 June 9, 2023 June 14, 2023	Corn Corn Corn	49 22 23

Specimen processing and slide-mounting. The wings, head, thorax, gaster, and/or male genitalia of the specimens were dissected, processed, and mounted in synthetic Canada balsam separately but on the same microscope slide following the protocol of Polaszek and Kimani (1990). In some cases, whole insects were mounted. Voucher specimens were deposited in the insect repository of the National Crop Protection Center, University of the Philippines Los Baños (UPLB), UPLB Museum of Natural History, and the National Museum of the Philippines.

Species identification. The specimens were identified to species level by consulting the keys and species descriptions of Nixon (1937) and Polaszek and Kimani (1990). However, the morphology of the male genitalia of *T. remus* in the original description was not described but an illustration, excluding the basal ring, was provided (Nixon 1937). This line drawing, along with that of the other species described therein and those illustrated by Polaszek and Kimani (1990), as well as the scanning electron micrographs of Masner (1980), were visually compared with the series of successfully slide-mounted male genitalia that may or may not have the basal ring attached.

To complement morphological identification, DNA barcoding was performed on a subset of the laboratory-reared population from Bocboc, San Carlos City (Pangasinan). The genomic DNA of adult *T. remus* was extracted using the GF-1 nucleic acid extraction kit (Vivantis Technologies) according to the manufacturer's protocol, with the addition of 8 ul of RNase per sample and an increase in centrifugation time from 1 minute to 5 minutes during the DNA precipitation step. Following DNA extraction, the mitochondrial cytochrome oxidase subunit 1 (*COI*), corresponding to the "barcode" region, was amplified using Taq DNA Polymerase (New England Biolabs, Inc) using primer pairs LCO1490 and HCO2198. The polymerase chain reactions (PCR) were conducted in Q-Cycler 96 Thermal Cycler (Hain Lifescience). The PCR conditions include an initial denaturation at 94 °C for 1 minute, followed by five cycles of 98 °C for 10s, 45 °C for 15s, 68 °C for 30s, and 35 cycles of 98 °C for 10s, 52 °C for 15s, 68 °C for 30s, as well as an extension at 68 °C for 5 minutes. The amplified DNA barcode was resolved in 1.2% agarose gel (Vivantis Technologies) and stained with Gel Red (Biotium) before viewing under UV light. Samples with positive amplification were sent to Macrogen (Macrogen Inc., South Korea) via Kinovett Scientific Solutions Co. (Quezon City, Philippines) for sequencing. The sequence chromatograms of each individual were checked, edited, and aligned to produce the consensus sequence using BioEdit Sequence Alignment Editor (Hall 1999). The nucleotide sequences were then compared with those deposited in GenBank using BLASTn (Altschul et al. 1990). This integrative taxonomy of combining a taxonomic key based on male genitalia with DNA barcoding using *COI* mitochondrial gene fragment is recommended for *Telenomus* species with their close morphological similarity (Wengrat et al. 2021).

Rearing of the host and parasitoid. The existing population of FAW at the Biocontrol Laboratory of the National Crop Protection Center, UPLB, originally collected from Gonzaga, Cagayan, was the source of egg-masses as host of the parasitoid. The larvae of FAW were reared on young leaves of corn until pupation. Pupae were pooled in Petri plates and when about to emerge, usually after seven days, the pupae were placed in an oviposition cage with 20% sugar solution dispensed in cotton balls inside a plastic plate and a few seedlings of corn in a bottle filled with water to maintain freshness served as oviposition substrates of female adults. Newly laid egg-masses of FAW on corn leaves were fastened onto a strip of paper in groups of 5-10 using a stapler and offered to ovipositing females of *T. remus* inside 500 ml Erlenmeyer flasks. The flasks were covered with two-ply paper towel kept in place using a rubber band. A cotton ball moistened with 20% sugar solution embedded inside a 1 ml Eppendorf plastic tube served as food to the adult parasitoids.

The strips of paper with stapled egg-masses of FAW were exposed for oviposition for 24 h to newly mated parasitoids. After 24 h exposure, the egg strips were removed and carefully cleaned with any parasitoids, placed into another Erlenmeyer flask for holding, and examined daily for emergence of parasitoids or host larvae. Neonates were trapped and removed from the flask to prevent cannibalizing the remaining parasitized eggs of *S. frugiperda*.

Strips of FAW egg-masses were exposed daily to adult parasitoids for oviposition up to four days after which the adult *T. remus* were preserved, coded, and kept as reference material. The cycle is repeated to maintain the parasitoids. Biological parameters observed were: number of parasitized egg-masses and eggs, duration of egg-adult period (in days), and sex ratio. The duration of egg-adult period was determined through daily observations from emergence to the adult stage of the parasitoids. All rearing was done at about 27 ^{0}C, 50-65% humidity, and photoperiod of 12:12 (L: D) h.

Documentation. Images of live and preserved specimens were captured using a Carl Zeiss Stemi 305 stereomicroscope (Zeiss Research Microscopy Solutions, Germany) equipped with a microscope camera (Axiocam ERc5s, Zeiss Research Microscopy Solutions, Germany) wirelessly connected to an iPad. The images were processed and annotated using an imaging software (Labscope version 2.8.1, Zeiss Research Microscopy Solutions, Germany).

For the slide-mounted specimens, several partially focused images were captured at intervals of 2 mm by using a phase-contrast compound microscope with an integrated high-definition camera (Primostar, Carl Zeiss; Jena, Germany). These images were stacked into a single focused image by using Zerene Stacker (professional edition, version 1.99, Zerene Systems LLC; Washington, USA) and the free software Combine ZP. Images of better resolution were saved and annotated.

Statistical analysis. Percentage parasitism between field and laboratory reared *T. remus* were analyzed statistically using t-test**.** Male to female sex ratio was computed based on emerged males and females.

RESULTS AND DISCUSSION

Field and laboratory parasitism. Field parasitism of egg-masses of *S. frugiperda* by *T. remus* was highest (80%) from Barangay Isabang, Tayabas, Quezon, lowest (10%) from Barangay Bocboc, San Carlos City, Pangasinan, and moderate (45.7 %) from Central Experiment Station, UPLB on corn (Table 2). Recently, in China, natural parasitism rates of *T. remus* in corn fields could reach up to 30% and 50% for egg-masses and eggs, respectively (Liao et al. 2021). Other studies showed that the parasitic capacity of *T. remus* on *S. frugiperda* egg-masses in the field was high (Ferrer 2001; Bueno et al. 2010; Pomari et al. 2013). Earlier, report of parasitism rates of *T. remus* on *S. frugiperda* reached 90% through inundative releases in corn fields in Venezuela (Ferrer 2001).

Under laboratory conditions, it was observed that *T. remus* adults readily parasitized freshly laid egg-masses (but not thawed frozen eggs) resulting in parasitism rates up to 87.5% for egg-masses and 83.1% for eggs of *S. frugiperda* originally collected from Barangay Bocboc, San Carlos City in Pangasinan. Reports show that *S. frugiperda* usually laid eggs in masses in one to three layers on leaf surfaces, usually covered with a layer of scales from female abdomen serving as physical barriers to parasitoids (Dong et al. 2021). However, *T. remus* can penetrate all the layers of the egg-mass resulting in 80-100% parasitization in laboratory studies (reviewed by Hay-roe et al. 2015), as was observed in this study (Table 2). Further, *T. remus* females could overcome the scale covering on egg-masses of *S. frugiperda*, are more aggressive, with higher searching capacity and proportion of parasitism when compared to *Trichogramma dendrolimi* and *T. pretiosum* (Dong et al. 2021). This is attributed to the co-evolution between *T. remus* and *Spodoptera* spp. The parasitoid females are attracted to the kairomone (z)-9-dodecene-1-ol acetate component of the sex pheromone of *S. frugiperda,* secreted by the accessory glands located at the abdominal tip of female moths and attached to the scale-hair cover of eggs during oviposition (Dong et al. 2021).

Sex ratio in *T. remus.* Based on observations, the male and female sex ratio of *T. remus* in the laboratory is 0.99: 1.01 for the Bocboc population (Table 2). On the average, for every one male individual, there is a female individual. This was translated to 87.5% egg-mass and 83.3% egg parasitism of FAW.

In the CES population, the sex ratio of the progenies is similar to Bocboc (0.97:1.03) with 100% egg-mass and 90.3% eggs parasitism of FAW. The sex ratio is an important biological parameter in assessing performance of *T. remus* for use in biological control program for *S. frugiperda* and should be in favor of the females since the females are involved in host selection and oviposition. In instances where there is preponderance of males, as in field collected parasitized egg-masses from Isabang (Quezon) (2:1) and Bocboc (1.3:0.70) (Table 2), this can be explained either by competition between *T. remus* females which causes imbalance in favor of the males.

Table 2. Some biological parameters of *Telenomus remus* Nixon collected from Luzon Island, Philippines.

Locality	Field Collected					Progeny			
	Total number of Egg- Masses	No. of Egg- Masses parasitized	% Parasitism		Sex ratio (M:F)	Egg-adult period (days)	% Parasitism		Sex ratio (M:F)
			Egg-Mass	Eggs			Egg-Mass	Eggs	
Barangay Isabang, Tayabas, Quezon	15	12	80	ND	2:1	ND	ND	ND	ND
Barangay Bocboc, San Carlos City, Pangasinan	50	5	10	47.8	1.3:0.7	9-10	87.5	83.1	0.99:1.01
Central Experiment Station, Pili Drive, UPLB College Laguna	94	43	45.7	85.7	0.7:1.3	11-12	100	90.3	0.97:1.03

Sex ratio between males and females from and Bocboc and CES (field collected and progeny) are not significantly different by t-test.

Species verification of *T. remus*. Morphological examination revealed that the species of egg parasitoid from the three collection sites is *T. remus* (Fig. 1 A-E). This identification is confirmed using molecular identification. The DNA barcode of the egg parasitoid, consisting of 633 bp, was successfully generated (GenBank Accession No. OR619425) (Fig. 2). This barcode sequence provides invaluable insights into the molecular identity of the parasitoid and can serve as a reference for future comparative studies. The sequence alignment revealed a significant percentage identity of 99.3% to 99.8% with *T. remus* (accession number MT906647.1), suggesting that the samples in Bocboc, San Carlos, Pangasinan is *T. remus* (Table 3). The generated DNA barcodes were uploaded in the GenBank and assigned accession numbers OR619417 to OR619426.

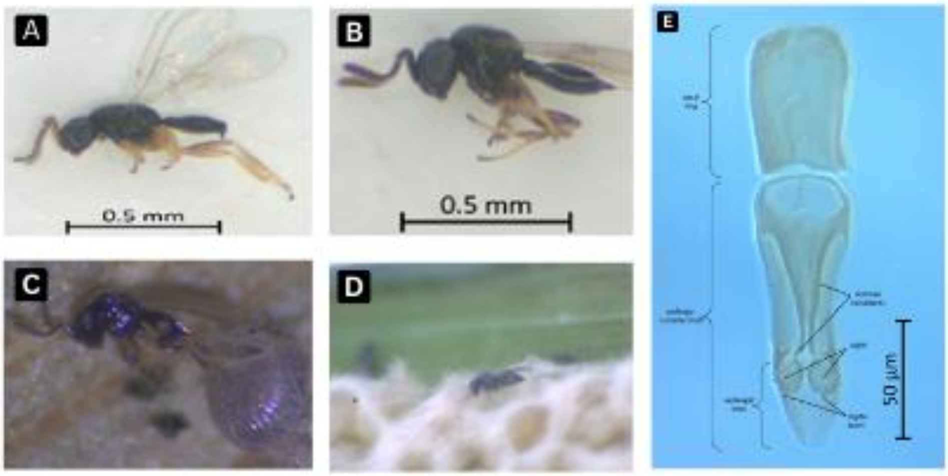

Fig. 1. *Telenomus remus* Nixon: (A) Male, (B) Female, (C) Newly emerged female, (D) Parasitized egg-mass with fully emerged adult, (E) Male aedeagus

Table 3. Summary of the most significant BLAST hit of the *cytochrome C oxidase I* (*COI*) barcode gene region of the *Telenomus remus* Nixon from laboratory-reared samples collected from Bocboc, San Carlos City, Pangasinan, Luzon, Philippines with the published reference sequence from GenBank.

Query Sequence	Query Length	Percentage Nucleotide Identity (%)	E-value	GenBank Accession Number	Description	Author/ Country
OR619417	611	99.3				
OR619418	611	99.3				
OR619419	611	99.3				
OR619420	611	99.3				
OR619421	611	99.3	0	MT906647.1	*Telenomus remus* mitochondrion, complete genome (1792 to 3309 of the gene region)	Li et al. 2021/ China
OR619422	633	99.8				
OR619423	633	99.8				
OR619424	633	99.8				
OR619425	633	99.8				
OR619426	633	99.8				

Fig. 2. DNA barcode of *Telenomus remus* Nixon (GenBank Accession No. OR619425) from laboratory-reared samples collected from Bocboc, San Carlos City, Pangasinan.

Metamorphosis in *T. remus*. The developmental stages of *T. remus* consist of the egg, two larval stages, pre-pupa, pupa, and adult male and female (Fig. 3). The durations of the egg to adult periods were 9-10 days for the Bocboc population and 11-12 days for the CES-UPLB (Table 2). The egg is a typical encyrtiform type (Fig. 3A) inserted within the FAW egg during oviposition, in which the egg stalk (slender portion, with an arrow) sticks out of the host chorion; light colored and viewed properly using phase contrast compound microscope. At 2 d after exposure to ovipositing female parasitoids, a minute first instar larva (Fig. 3B) appeared globular, white, and transformed into a bigger and cylindrical second instar larva at 3 d (Fig. 3C). At 4 d (Fig. 3D), the second instar larva had consumed the host egg contents and excreted the meconium and is called pre-pupa. At 5d, the pre-pupa transformed into a pupa (Fig. 3E), which is light brown initially. At 6d to 8d (Fig. 3F to H) the male pupa blackened, visibly exarate and a male adult emerged at 9d (Fig. 3I). The female adult emerged at 10d (Fig. 3J), a day after the male adult. It was observed that only one adult *T. remus* emerged from a parasitized egg of *S. frugiperda* indicating that it is a solitary parasitoid, although superparasitism was also observed. These observations are consistent with previous reports of others working on *T. remus* as reviewed by Cave (2000).

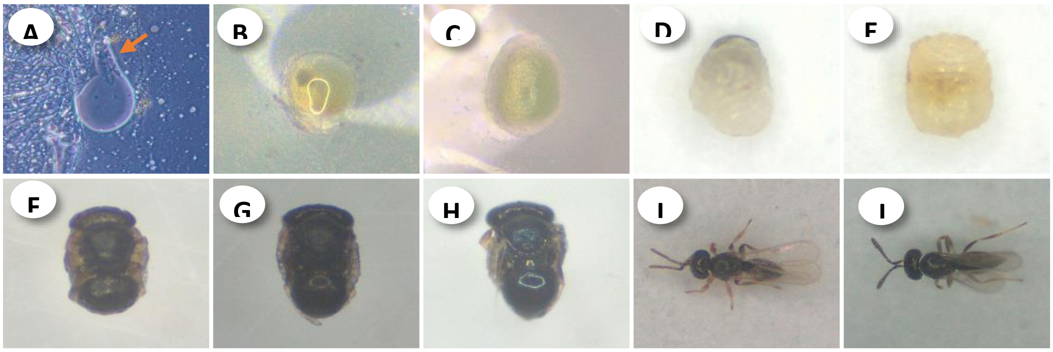

Fig. 3. Egg to adult periods *of Telenomus remus* Nixon from parasitized eggs of *Spodoptera frugiperda* (J.E. Smith): corresponding to the egg (A), first instar larva (B), second instar (C), late second instar with meconium, called pre-pupa (D), early pupal stage (E), advanced pupal stages(F,G,H), newly emerged adult male (I), and female (J). (Photo credits: M.D. Javier).

CONCLUSION

Integrative taxonomy and biological characterization in this study showed that a *Telenomus* species found parasitizing *S. frugiperda* eggs in the Philippines is *T. remus.* This first report lays the foundation for the inclusion of *T. remus* as an IPM tool against this pest in the country. Future studies should focus on the mass-rearing of *T. remus* on alternative hosts including field validation and assessment for cost effective mass production.

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LIFE CYCLE ASSESSMENT OF BROILER PRODUCTION IN TUNNEL-VENTILATED AND CONVENTIONAL POULTRY HOUSES IN GENERAL AGUINALDO, CAVITE, PHILIPPINES

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ABSTRACT

The growing demand for chicken meat necessitates the expansion of the poultry industry. To address production challenges, technology is being increasingly adopted, especially to mitigate the effects of climate change. However, the industry's rapid growth raises concerns about its sustainability, with intensive poultry farming leaving a significant ecological footprint. Notably, research in the Philippines has not adequately addressed the environmental performance of broiler production. This study aims to bridge this gap by conducting a comparative environmental impact assessment of conventional and tunnel-ventilated poultry houses in General Aguinaldo, Cavite. Life cycle assessment was done to evaluate the environmental impact of both types of poultry houses. The system boundary of the study is the operation involving broilers from brooding to post-brooding. The environmental impacts include global warming potential (GWP), eutrophication potential (EP), and acidification potential (AP). Results of the study showed that tunnel ventilated system had higher GWP and EP while conventional production had higher AP per harvested live chicken. The use of renewable energy sources, proper orientation of poultry houses, rainwater harvesting, efficient water quality management, and adding adsorbents to reduce odor emitted by poultry manure were recommended to minimize environmental impacts.

Key words: life cycle assessment, system boundary, global warming potential, eutrophication potential, acidification potential

INTRODUCTION

Chicken consumption has witnessed a remarkable surge worldwide, becoming a staple in the diets of people from diverse cultures and regions. The growing global population, changing dietary preferences, affordability, and ease of production and distribution have all contributed to the increasing popularity of chicken meat. According to recent statistics, chicken is now the most consumed meat globally, surpassing beef and pork (FAO 2021). This shift in consumer behavior has significant implications for the poultry industry, global food systems, and sustainability considerations.

In the Philippines, the poultry industry ranked third as one of the most significant contributors to the country's agriculture. In 2021, the total chicken production was estimated at 1.74 million metric tons, liveweight. It shared 12.5 percent of the total agricultural output. The total chicken population in the country stood at 190.74 million heads for the last quarter of 2021. At current prices, the gross value of poultry production amounted to PhP 193.48 billion, or 7.7 percent higher than the earnings in 2020 (PSA 2021). Chicken meat is the second most popular meat in the Philippines, following pork. Broiler chicken, mainly for meat production, accounts for 34 percent of the total chicken inventory. This is 5.5 percent higher than the previous year (PSA 2021). At present, chicken broiler production has become one of the most progressive animal enterprises in the country. This is evident with backyard enterprises shifting to form large integrated contract farming operations. Most broiler entrepreneurs venture into contract growing, technically in collaboration with large private companies that provide them the chicks, feeds, technical services, and the market for the products.

In order to increase production efficiency, new technologies have been employed in the poultry industry. The livestock industry, through which it is included, is among the most technology-intensive in the agriculture sector. For the past years, there has been a wide application of technology in its operations spanning from farmhouses establishment, production operations, and even product packaging and tracking. In effect, technology enables farm operations to become more mechanized, resulting in the expected time and labor savings, reduced input costs, reduced production periods, and significant increase in productivity.

Climate change poses a significant challenge for the global animal industry, impacting the performance and well-being of animals, particularly chickens. Chickens are particularly vulnerable to heat stress, and their productivity is highly dependent on narrow temperature ranges. A study by Kang et al. (2020) examined the effects of rapid versus gradual temperature increases on laying hens and revealed that rapid temperature elevations had more severe consequences, particularly in terms of mortality rates. In a related study, Liang et al. (2020) conducted a review of sprinkler technology as a physical intervention to mitigate heat stress in broilers, highlighting its positive impact on cellular metabolism and its potential to counteract heat stress effects. Additionally, a study by Kim et al. (2020) investigated the influence of heat stress on laying hens exposed to varying temperature conditions. Hens subjected to high temperatures exhibited elevated rectal temperature, heart rate, and infrared surface temperature readings throughout the study, emphasizing the challenges chickens face when exposed to rapidly rising temperatures. These studies underscore the need to regulate temperature increases within poultry housing systems.

Due to the varying climatic conditions, technological advances related to environmental control to provide suited conditions necessary for a specific life stage of the livestock became of critical importance. In the poultry industry, mechanical ventilation systems have gained significant attention for years owing to the growing need to regulate the environmental parameters necessary for production. Ventilation in livestock shelters controls several environmental factors by diluting inside air with outside air (Holmes 1989). A proper ventilating system must perform three essential functions, namely: bring in fresh air into the building through planned openings; thoroughly mixes outside and inside air; pick up heat, moisture, and air contaminants; lower temperature, humidity, and contamination levels; and withdraw moist and contaminated air from the building.

There are various types of poultry house ventilations. In the Philippines, natural or conventional ventilation is widely used. Natural or conventional ventilation relies on opening the house to the right extent to allow outside breezes and inside convection currents to blow air into and through the house. This is often done by lowering or raising sidewall curtains, flaps, or doors. Natural ventilation is often referred to as "curtain ventilation". This ventilation works well only when outside conditions are near desired in-house conditions.

On the other hand, in recent years, tunnel ventilation has emerged in order to mitigate thermal stress. This system is defined by a linear arrangement of air inlets and outlets, with air inlets situated at one end and exhaust fans positioned at the opposite end (Sarentonglaga et al. 2019). Tunnel ventilation aims to keep animals comfortable in warm to hot weather by using the cooling effect of high-velocity airflow. The tunnel setup is suited to warmer areas. Tunnel systems are designed to handle the expected need for heat removal and provide a condition for air exchange and let out excess house heat in hot weather. The tunnel setup also provides wind-chill cooling, moving air as in a wind tunnel through the length of the house.

Based on the Bureau of Animal Industry (BAI), there were 36 poultry farms registered in 2010, which increased to more than seven times in 2016, for a total of 269 farms. However, only 33 and 244 farms were located based on BAI registration in 2010 and 2016, respectively. Of the 33 farms registered in 2010, 23 use natural or conventional ventilation. The majority of those registered in 2016, or 163 farms of the 244 farms, are still into natural or conventional ventilation. On the other hand, there is also a growing adoption of tunnel ventilation technology in the poultry industry. Based on the BAI registration, poultry farms using the technology increased from 8 farms in 2010 to 81 farms in 2016.

With the growing demand for chicken meat, it is evident that the poultry industry will continue to expand in the foreseeable future. This expansion will be accompanied by the ongoing adoption of technological solutions to address production challenges, particularly those arising from the effects of climate change. However, as poultry operations increase in size, concentration, and intensity, concerns about the industry's sustainability have become more pronounced. During the past years, the environmental impact of the poultry industry has received ever-growing attention. Intensive poultry farming leaves a substantial ecological footprint, posing significant risks to both the environment and human health, demanding effective management practices. The poultry industry and its by-products contribute to NH_3, N_2O, and CH_4 emissions, impacting global greenhouse gas levels and the well-being of both animals and humans. Poultry litter and manure may contain contaminants like pesticide residues, microorganisms, pathogens, pharmaceuticals (including antibiotics), hormones, metals, and imbalanced macronutrient ratios, resulting in air, soil, and water pollution and the development of drug-resistant pathogenic strains. The release of dust from intensive poultry operations, containing feather and skin fragments, fecal matter, feed particles, microorganisms, and other pollutants, adversely affects poultry health, as well as the health of farm workers and nearby communities. Persistent odors compound these concerns, negatively affecting worker health and community well-being (Gržinić et al. 2023). In light of these issues, the broiler industry faces the critical challenge of achieving a balance between meeting the growing population's demands for increased production and the imperative to minimize its environmental impact for long-term sustainability.

Recent research on broiler production in both tunnel-ventilated and conventional poultry facilities within the Philippines has predominantly addressed grower satisfaction (Franco 2021), enhancements to cooling systems (Ballaran and Victoria 2019), the quantitative evaluation of biosecurity practices in broiler farms (Tanquilut et al. 2020), and considerations of productivity and financial viability (Salas et al. 2016). However, there remains a notable gap in the literature, with no existing study evaluating the comprehensive environmental performance of broiler production. Consequently, this study aims to rectify this gap by conducting an environmental impact assessment of broiler production, driven by the increasing adoption of tunnel ventilation technology in the Philippines. In light of this trend, a comparative analysis between this emerging technology and conventional practices was conducted, given expectations that the reliance on tunnel ventilation will continue to grow as a significant adaptation measure to address climate variability and unpredictability arising from climate change.

To facilitate a better understanding of the environmental impacts of broiler production, life cycle assessment (LCA) was employed to determine the overall environmental performance of broiler production. General Aguinaldo, located in Cavite, was selected as the study area due to its status as one of the major broiler producers in the country. This region also exhibits a widespread adoption of tunnel ventilation technology and substantial number of poultry farms using conventional poultry housing. This will ensure the results of the analysis for the types of poultry houses to be readily comparable due to the consistent environmental conditions. Now, this study aimed to identify as well as quantify the various environmental impacts of the commercial broiler production using tunnel ventilated house and conventional house in General Aguinaldo, Cavite.

METHODOLOGY

This study used the LCA methodological framework established by ISO 14040. It involves four phases, namely, the definition of goal and scope, life cycle inventory, life cycle impact assessment, and interpretation of the results.

Goal and scope of the study. The goal of the study is to analyze the environmental performance of broiler production between conventional and tunnel-ventilated poultry houses. Specifically, the system boundary of the study is the operation involving broilers from brooding to post-brooding. Inventory analysis was conducted in the facility of purposively selected poultry farm co-operators in General Emilio Aguinaldo, Cavite. There were four conventional and five tunnel ventilated houses investigated for this study. There were two farms that had two conventional houses each while all of the tunnel ventilated houses are located in one farm. The functional unit used in the study was per harvested animal unit. The impact categories include global warming potential (GWP), atmospheric acidification potential (AP), and eutrophication potential (EP).

Inventory analysis, This phase involves identifying and quantifying the inputs and outputs of the production process as covered in the system boundary. At this stage, the following were quantified: the number of poultry animals, feeds, volume of water required for each of the life cycle stages, volume of diesel used for transportation, electricity consumption, amount of liquified petroleum gas (LPG) used for warming the air inside the poultry houses, amount of solid wastes, excess nitrogen (N) and phosphorus (P), methane (CH_4), carbon dioxide (CO_2) and sulfur dioxide (SO_2) emissions.

The quantity of animals, amount of feeds, water, diesel and LPG were derived from farm records and interviews with the farm managers and operators. The electricity consumption was measured in kilowatt-hour (kWh) which will be obtained by determining the power (in Watts) of the light bulbs, fans, feed motor, and water pump and other electrical equipment used during the production and the time this equipment are used. The product of the power and the time used provides the electrical energy required for each of the equipment. In conventional houses, the estimate of water usage per house was calculated by determining the number of waterers used and frequency of refilling them. The data on water consumption in tunnel ventilated buildings, on the other hand, were collected from the LCD screen of the monitor of the automatic control system verified thru water meter readings. Daily electric power consumption in conventional and tunnel ventilated house was derived from the readings on designed microcontroller-based power consumption logger and kilowatt-hour meters, respectively.

The estimation of amount of the manure was based from results from the study of American Society of Agricultural and Biosystems Engineers (ASABE) (2010) wherein it was stated that on the average, 0.23 lb of manure is excreted by one broiler per day (Ni and Heber 2013). The volume of wastewater was approximated by applying the values recommended by Economopoulos (1996) which 37.5 m3 of wastewater per 1000 birds in poultry processing. Since such numbers were derived from the whole life cycle of poultry production starting from its initial stage until meat processing, it is important to note that integrating these to this study provides an overestimate of the amount of wastewater that might have been discharged in the actual production since the boundary was set only to brooding to post-brooding in broiler production.

The Total N and total P of water were analyzed before and after the operation. Water samples was submitted to an analytical laboratory for the total N and total P analyses. Composite sampling was employed, i.e., water samples were collected from outlets of the farmhouses before the analysis. The total N and P was analyzed in three replicates. Excess N and P was calculated using Equation 1:

$$\text{Excess N and P} = O_{NP} - I_{NP} \qquad \text{Equation 1}$$

Where:

I_{NP} is the N and P content of the water before the operation, and O_{NP} is the N and P content of the water after harvesting.

Impact assessment. The impact categories that were applied are global warming potential (GWP), atmospheric acidification potential (AP), and eutrophication potential (EP). These were derived using the results of the inventory analysis. The GWP of a substance is the ratio between the contribution to the heat radiation absorption resulting from the instantaneous release of 1 kg of greenhouse gas and an equal emission of CO_2 integrated over time (Heijungs 1992). The GWP of CO_2 and CH_4 expressed as equivalent over a 100-year life span. The GWP characterization factor of CO_2 is 1.00 (Houghton et al. 1994), while CH_4 is 28.00 (IPCC 2014).

$$\begin{aligned}\mathrm{CO_2\ emission, kg} = {} & \left[(\text{Electricity consumption, kWh})\left(\frac{0.53606\ \mathrm{kg\ CO_2}}{\mathrm{kWh}}\right)\right] \\ & + \left[(\text{Volume of diesel, L})\left(\frac{2.778\ \mathrm{kg\ C}}{3.74\ \mathrm{L\ diesel\ fuel}}\right)(0.99)\left(\frac{44}{12}\right)\right] \\ & + \left[(\text{Weight of LPG, kg})\left(\frac{3.03\ \mathrm{kg\ CO_2}}{\mathrm{kg\ LPG\ fuel}}\right)\right] \\ & + \frac{\left[\begin{gathered}(\text{Average daily gain (g)})(\text{ Total population})\left(\frac{0.002946\ \mathrm{g}}{\mathrm{hour}}\ \mathrm{CO_2}\right) \\ x\ (\text{Total Hours of Broiler Production})\end{gathered}\right]}{\frac{1000\ g}{1\ kg}}\end{aligned} \qquad \text{Equation 2}$$

$$CH_4\ of\ Manure_{\mathrm{a}} = (Pop_{\mathrm{a}})(EFmanure_{\mathrm{a}}) \qquad \text{Equation 3}$$

Where:
$\mathrm{CH_4}$ of $\mathrm{Manure_a}$ = methane emission from manure management (Gg/year)
$\mathrm{Pop_a}$ = population (in 1000 heads)
$\mathrm{EFmanure_a}$ = emission factor (kg CH_4 /head/year)

$$\begin{aligned}\mathrm{GWP} = {} & (\mathrm{CO_2\ emission})(\text{GWP characterization factor}) \\ & + (\mathrm{CH_4\ emission})(\text{GWP characterization factor})\end{aligned} \qquad \text{Equation 4}$$

The AP is based on the emissions of SO_2, an acidifying substance to the air. SO_2 emission was computed using Equation 5. The electricity consumption was converted to an equivalent volume of diesel in liters using the conversion factors 3.6 megajoule (MJ) which is the available for every kilowatt-hour of electricity, and 36 MJ which is the heating value of one liter of diesel fuel (Korres 2013). The resulting value was added to the actual volume of diesel used for transportation. The total volume of diesel was multiplied by 0.850 kg/L, which is the density of diesel fuel based on the Philippine standards, 0.05%, which is the estimated sulfur content in diesel (Philippine Clean Air Act of 1999), and 64/32, which is the ratio of molecular weights of sulfur dioxide and sulfur, respectively (Abasolo and Zamora 2016). The weight of LPG was multiplied by 0.014%, which is the estimated sulfur content in LPG (PNS/DOE QS 005:2016), and by the same molecular weight ratio as before. AP was calculated using Equation 6. The AP factor of SO_2 is 1.00 (Heijungs 1992).

$$\begin{aligned}&\mathrm{SO_2\ emission, kg}\\&=\left[(\text{Electricity consumption, kWh})\left(\frac{3.6\text{x}10^6\ \text{J}}{\text{kWh}}\right)\left(\frac{1\ \text{L diesel fuel}}{36\text{x}10^6\ \text{J}}\right)\right.\\&\left.+\ (\text{Actual volume of diesel used, L})\right]\left[\left(\frac{0.850\text{kg}}{1\ \text{L diesel fuel}}\right)(0.05\%)\left(\frac{64}{32}\right)\right]\\&+\left[(\text{Weight of LPG, kg})(0.014\%)\left(\frac{64}{32}\right)\right]\end{aligned}\qquad \text{Equation 5}$$

$$\text{AP of SO}_2 = (\text{SO}_2\ \text{emission})(\text{AP factor}) \qquad \text{Equation 6}$$

The EP converts the inventory data to phosphate equivalents. This is calculated based on the excess N and P using Equation 7 where m_i is the amount of N and P released to soil and water. The EP factors of N and P are 0.42 and 3.06, respectively (Heijungs 1992).

$$\text{EP} = \sum_i (\text{EP factor}_i)(\text{m}_i) \qquad \text{Equation 7}$$

Interpretation. In this final phase of LCA, the most significant environmental impact category and the life cycle stage were identified based on inventory analysis and impact assessment results. Conclusions and recommendations were also formulated based on the same results.

RESULTS AND DISCUSSION

Broiler production. Broiler production is divided into two stages: brooding and post-brooding. The animals stay in the broiler house for more than 30 days, from brooding to post-brooding, until they are ready for harvesting. Brooding is the mechanism of providing young chickens with heat after hatching up or when they are a day old until their natural heat-regulating system becomes fully developed, usually when they are 14 days old. During the brooding stage, for this study, one conventional farm used an infrared heater suspended about 80 cm from bird height to warm the in-house air or the surrounding of the broiler chicken, while the other conventional farm provided heat to the chickens by burning charcoals inside a metal container hanged approximately 60 cm above bird level. Both conventional farms used coffee pulp as floor litter to aid in the brooding process. Meanwhile, the tunnel-ventilated house uses a direct-fired gas heater to heat the air and old newspapers as floor cover during brooding. Infrared and direct-fired gas heaters use liquefied petroleum gas (LPG) tanks for combustion.

To ensure that the chickens are comfortable not only during brooding but also throughout the remaining production period, one factor that is maintained is proper ventilation. Conventional broiler house employs natural ventilation wherein air exchange happens as a result of temperature and pressure differences inside and outside the house. During the whole course of broiler production, the conventional broiler house is in natural ventilation mode. Flock man manually adjusts sidewall curtains to take advantage of natural ventilation depending on the ambient air condition, which is mainly influenced by temperature. However, the flock man does not have any measuring device to determine the temperature and humidity levels inside the house, and the adjustment of the curtain depends solely on the flock man's observation of the chickens' behavior or response to the thermal condition of the surrounding air. For instance, if the flock man feels that the air is too cold for the chickens, he will roll up or fully close the curtains, and if it is too hot, the curtains will be rolled down or fully open. Nevertheless, in general, curtains are fully closed during the brooding stage and are gradually rolled down starting from post-brooding at the age of 15 days until they are fully open at the last week of production.

Aside from the manual adjustment of curtains in a conventional house, a large part of the labor handled by the flock man is the manual refilling of feeders and waterers. Trough and round feeders and galloners are used when the birds are still small, and when they grow older and are bigger in size, trough feeders are pulled out while round feeders are retained, and all of the galloners are replaced with round pan waterers. Meanwhile, since in-line feeders and nipple drinkers are fully automatic, the only labor needed in the feeding and watering system of the tunnel-ventilated house is the manual refilling of feed hoppers and preparation of antibiotics, vitamins, or other medicine to be mixed with water to be supplied to the birds.

On the other hand, there are three stages of ventilation modes applied in tunnel-ventilated broiler houses: minimum, transitional, and tunnel ventilation (Aviagen 2019). These ventilation modes are set and controlled using automatic control systems. Minimum ventilation is set from the first day of growing until the last part of the brooding stage, wherein direct-fired gas heaters, sidewall inlets, and exhaust fans intermittently operate based on the set threshold temperature and humidity values. After which, transitional ventilation, as the term implies, is the ventilation setting amid the transition period from brooding to post-brooding production. This setting is performed to prepare the homeothermic capacity of chicks to adapt to the shift from a warm to a cool in-house environment. In transitional ventilation, alongside the sidewall exhaust fans, one to two tunnel exhaust fans are also running to withdraw the mixture of stale air and air coming from the sidewall and tunnel inlets. There will come a time when one or two tunnel exhaust fans will not be sufficient to withdraw the heat or cool the inside of the poultry house because the amount of heat in the building will increase since the chickens are also emitting more body heat due to gain in mass as the production progresses. During such times or hot weather, the broiler house is set to tunnel ventilation mode. In the tunnel ventilation setting, the tunnel exhaust fans withdraw the stale air, and fresh air enters the tunnel inlets located at the opposite end of the tunnel fans. All sidewall inlets are closed when the house operates in tunnel ventilation mode. Evaporative cooling pads are also switched on with the tunnel inlets and fans if the exhaust fans are insufficient to bring the in-house air to the desired cool temperature.

The conventional house had no sidewalls, rather the house was only covered by polyester plastic curtains during brooding and cold periods. In contrast, the side wall of tunnel-ventilated houses is either made up of concrete or sandwich panel polyurethane, depending on the owner's preference. The conventional house, which is elevated has cylindrical concrete columns and bamboo posts to support the structure of the house. It means that chicken manure and other animal excretions passing through the spaces between bamboo slats are accumulated on the soil surface under the conventional house. In contrast, the concrete floor of tunnel-ventilated houses is not elevated or is directly connected to the ground resulting to all excreted elements being amassed in the concrete basement below the plastic slats flooring.

There were four conventional and five tunnel-ventilated houses investigated for this study. Two farms had two conventional houses each while all tunnel-ventilated houses were located in one farm. The capacities of the conventional house were 2,450, 4,000, 5,000, and 6,600 birds. Only one tunnel-ventilated house had a 32,000 bird capacity, while each remaining house had 25,000 birds. It can be noticed that tunnel-ventilated houses can accommodate five to seven more birds per square meter than a conventional one. For the conventional type, 18,009 day-old chicks were distributed to four houses, while 135,072 day-old chicks were placed accordingly in five houses for the tunnel-ventilated facilities. After the brooding stage, 17,196 chickens remained in the conventional facilities, while there were 133,890 chickens in the tunnel ventilated confinement. The final population for the two types of houses come harvest time was 16,890 and 128,567 live chickens, respectively. Table 1 compares the characteristics of the two types of broiler houses.

Table 1. Broiler house design, structure, and management practices

Item	Conventional	Tunnel Ventilated
House floor dimensions width x length (m), capacity per storey, stocking density (birds/m2), number of storeys	11 x 60, 6600, 10, 1	12 x 122, 25000, 17, 1
	11 x 35, 4000, 10, 1	12 x 122, 25000, 17, 1
	10 x 48, 5000, 10, 1	12 x 122, 25000, 17, 1
	10 x 24, 2450, 10, 1	12 x 122, 25000, 17, 1
		15 x 137, 32000, 15, 1
Housing type	Elevated, open sided with lumber on concrete columns	Non-elevated, closed housing
Roof type	Corrugated GI sheets without insulation, monitor type with wood trusses	Corrugated GI sheets with insulation,
		gable/double span type with steel trusses
Floor type	Bamboo slats	Plastic slats mounted on concrete
Sidewalls	None	Concrete or sandwich panel polyurethane
Brooding set-up	Infrared heater or charcoal; coffee hull mounted on double-layer net overlaid on bamboo slats	Direct-fired gas heater; old newspaper overlaid on plastic slats
Ventilation system	Natural ventilation; manual adjustment of tarpaulin curtains	Combination of minimum, transitional, and tunnel ventilation; evaporative cooling pads; exhaust fans; automatic controls
Feeding system	Ad libitum feeding using trough and round feeders	Ad libitum feeding using automatic
		in-line feeders
Watering system	Ad libitum drinking using galloners and round pan with grills	Ad libitum drinking using automatic in-line nipple drinkers

Inventory analysis. In order to make values comparable, data were normalized per 10,000 birds. The inventory revealed that about 10,553 kg of feeds and 6,343 liters of water were consumed while using 203 kWh of electrical energy were used in conventional production. On the other hand, 8,614 kg of feeds, 18,406 liters water, and 1,127 kWh electrical energy were expended to come up to a same harvest population in the tunnel-ventilated houses. It can be seen in Table 2 that values increased significantly in post-brooding periods as broiler chickens need more feeds and water as they grow.

Electricity consumption did not differ much in conventional poultry houses during the brooding and post-brooding stages. This is because they only rely on natural ventilation to regulate the temperature inside the poultry house. Meanwhile, electricity consumption in tunnel-ventilated poultry houses increased more than double in the post-brooding stage. During this stage, broiler chickens need proper ventilation to lower temperature because their feathers inhibit heat loss. Tunnel-ventilated poultry houses consume higher electrical energy because of the tunnel and sidewall fans, the pump for the evaporative cooling system, the water pump for the drinking line, and another pump for the feeder line, in contrast to the conventional setting where the light bulbs and fluorescent lamps are only the electrical fixtures used during the production since the water and feeds are manually provided to the animals through human labor.

The inventory also shows that about 1,391.02 kg CO_2, 0.1526 kg SO_2, and 0.0096 kg CH_4 were emitted in conventional ventilation settings. On the other hand, 1,488.57 kg CO_2, .1502 kg SO_2, and 0.0088 kg CH_4 were the emissions calculated for the tunnel-ventilated setting. Higher emissions were obtained in the tunnel-ventilated facilities due to more intensified production and electrical equipment use. In conventional poultry houses, the brooding stage has higher CO_2 emissions because of LPG fuel consumption to heat the facility's air. LPG is not used during the post-brooding stage because the animals by that time require cooler temperature as they age. On the other hand, the post-brooding stage in tunnel-ventilated poultry houses still had higher CO_2 emissions despite the non-use of LPG. Higher emission in the said stage is due to the respiration of broiler chickens. Furthermore, it was found that conventional production released 7,916 kg of manure and176,896 liters of wastewater which is lower when compared to tunnel-ventilated production, which released 7,979 kg of manure and 179,254 liters of wastewater.

Results from the laboratory analyses detected that the total N and P in conventional had smaller concentrations, 94.9 mg/L, and 65.3 mg/L, compared with the values in the tunnel ventilated production, which are 368.65 mg/L and 101.72 mg/L, respectively. This may be because lesser manure flows out of the conventional poultry house system than the tunnel-ventilated poultry house. Moreover, this could be attributed to the presence of wastewater in conventional systems, including the water utilized for cleaning bamboo slats or floors, and minimal solid waste because a large part of the bulk volume of the manure passes through the space between the slatted floors and are accumulated on the soil beneath the elevated flooring of the house. In contrast, the wastewater in the tunnel-ventilated system is more concentrated with solid waste as all of the accumulated manure is not released out of the effluent pipe until the last day of harvest.

Table 2 summarizes all the inputs and outputs for broiler production in conventional and tunnel-ventilated poultry houses per 10,000 birds.

Table 2. Inventory of input and output values for broiler production per 10,000 birds.

Input	Conventional	Tunnel Ventilated	Output	Conventional	Tunnel Ventilated
Brooding (Day 1-14)					
Feeds (kg)	2,166	1,892	CO_2 (kg)	862.05	716.85
Water (L)	1,328	5,677	SO_2 (kg)	0.0932	0.0656
Electricity (kWh)	94	311			
Diesel for transportation (L)	99.95	31.09			
LPG (kg)	305.40	185.09			

Input	Conventional	Tunnel Ventilated	Output	Conventional	Tunnel Ventilated
Post-brooding (Day 15-30)					
Feeds (kg)	8,387	6,722	CO_2 (kg)	528.97	771.72
Water (L)	5,015	12,729	SO_2 (kg)	0.0594	0.0846
Electricity (kWh)	109	816	CH_4 (kg)	0.0096	0.0088
Diesel for transportation (L)	118	35.85	Manure (kg)	7,916	7,979
			Wastewater (L)	176,896	179,254
			Total N (mg/L)	94.9	368.65
			Total P (mg/L)	65.3	101.72

Impact analysis. The potential environmental impacts of broiler production for both conventional and tunnel-ventilated poultry houses are presented in Table 3. The results showed that broiler production in the tunnel-ventilated house has higher global warming potential with 40,036.67 kg CO_2 equivalent and acidification potential value of 4.03744 SO_2 kg equivalent because of its higher electricity consumption. Tunnel-ventilated broiler houses consume higher electrical energy because of tunnel and sidewall fans, a pump for the evaporative cooling system, a water pump for the drinking line, and another pump for the feeder line. Meanwhile, in the conventional setting, light bulbs and fluorescent lamps are the only electrical fixtures used during production since water and feeds are manually provided to the animals through human labor.

Higher eutrophication potential in the tunnel-ventilated house is also reported with 2,247.18 kg PO_4^{3-} equivalent, wherein higher concentrations of total N and total P values were present in the wastewater from the tunnel-ventilated house. This implies that broiler production in tunnel-ventilated poultry houses generates higher amount of nutrients that could lead to excessive plant growth and oxygen depletion in the receiving water bodies. In the rapid health impact assessment of a proposed poultry processing plant conducted by Baskin-Graves at al. in 2019, the growth of toxic microorganisms such as *Pfiesteria piscicida* was impelled by eutrophication associated with chicken manure. This microorganism has been found to cause temporary memory loss, immunosuppression, decreased cognitive function in exposed populations, respiratory problems and eye irritation, gastroenteritis, headaches, and fatigue. Excess and N and P can also seep into groundwater during water runoffs and can be highly toxic to fish and humans when dissolved in water due to its high ammonia level (Keena and Meehan 2022). Aside from the eutrophication potential caused by the higher concentration of N and P, it was found in the study of Nowak et al. in 2016 that long-term exposure and inhalation of odorous compounds from wastewater and chicken manure demonstrated marked cytotoxic effects and acute cell damage to people.

Table 3. Environmental impact potentials of broiler production between conventional and tunnel ventilated poultry house.

STAGE	GWP kg CO_2 equiv		EP kg PO_4^{3-} equiv		AP SO_2 kg equiv	
	Conventional	Tunnel Ventilated	Conventional	Tunnel Ventilated	Conventional	Tunnel Ventilated
Brooding	3,104.95	19,365.33	151.81	2,247.18	0.335815	1.77185
Post-Brooding	1,883.61	20,671.33			0.21148	2.26559
Total	4,988.56	40,036.67	151.81	2,247.18	0.547295	4.03744
Equivalent per harvested live chicken	0.30	0.31	0.01	0.02	3.240e-05	3.140e-05

Note: GWP (global warming potential); EP (eutrophication potential); AP (acidification potential)

In order to make the values comparable, the equivalents per harvested live chicken were computed. The tunnel-ventilated system was also higher in terms of the equivalent GWP and EP per harvested live chicken with 0.31 kg CO_2 equivalent and 0.02 kg PO_4^{3-} equivalent, respectively. Interestingly, it can be noticed that conventional production had higher AP per harvested live chicken with 3.240e-05 SO_2 kg equivalent because of the higher amount of LPG fuel consumed per bird during the brooding period using such type of ventilation. Higher acidification potential leads to acidic deposition, which acidifies the soil, surface waters, and groundwaters and affects nutrient cycling. The sulfur accumulation in the soil can reduce plants' potential uptake of nutrients and water. High acidity can also reduce the diversity and abundance of aquatic species in nearby waters. Aside from the discharge of very acidic groundwater, it can also cause water pipes corrosion, leading to leakage and high heavy metal content in drinking water. Nevertheless, there is very little difference between the equivalent GWP, EP, and AP per harvested live chicken between the two types of poultry houses. It must be noted that these values can only be applied to the specific design of poultry houses and management practices mentioned in Table 1 with consideration to the climatic condition of the Philippines.

The percent contribution to the environmental impact potentials is shown in Table 4. In conventional poultry houses, the brooding stage has a higher GWP (62.24%) because of the consumption of LPG fuel to heat up the air inside the facility. LPG is not used during the post-brooding stage because the animals by this time require cooler temperatures as they age. On the other hand, the post-brooding stage in tunnel-ventilated poultry houses still had higher GWP (51.63%) despite the non-use of LPG during the brooding stage. Higher GWP in the said stage is due to high electricity consumption.

Moreover, methane emission from manure management was only accounted for in the post-brooding stage. The volume of manure generated by broiler chickens in tunnel-ventilated poultry houses was very high compared to its counterpart. This also adds up to higher GWP in the post-brooding stage of broiler production in tunnel-ventilated houses. Regarding the acidification potential (AP), the brooding stage in the conventional poultry house also had a higher value (61.36%). Again, this is due to the consumption of LPG during the said stage. On the other hand, higher AP (56.11%) in the post-brooding stage in the tunnel-ventilated system is due to its high electricity consumption. The percent contribution per stage of the eutrophication potential was not computed since laboratory analyses of water samples were done only on the last day of harvest.

Table 4. Percent contribution to the environmental impact potentials

Stage	**GWP kg CO_2 equiv**		**AP SO_2 kg equiv**	
	Conventional	**Tunnel Ventilated**	**Conventional**	**Tunnel Ventilated**
	Percent			
Brooding	62.24	48.37	61.36	43.89
Post-Brooding	37.76	51.63	38.64	56.11

Interpretation. The environmental characterization of broiler production in conventional and tunnel-ventilated poultry houses identified the stage with the highest contributions to the selected impact categories. For the conventional poultry house, the brooding stage has the highest global warming potential and acidification potential due to the consumption of LPG. On the other hand, broiler chickens production in tunnel-ventilated poultry houses showed the highest global warming potential and acidification potential during the post-brooding stage due to its high electricity consumption. The highest eutrophication potential was seen in broiler production in tunnel-ventilated poultry houses due to the release of higher volumes of wastewater with more concentrated solid waste.

Given these findings, improvements should be made to minimize the use of electricity and LPG. Renewable energy sources like solar energy, one with the highest potential among the renewable sources, can be considered to reduce emissions. Solar energy can be used to convert solar radiation into electricity using photovoltaic (PV) technology. This can be done by mounting solar panels on the roof of poultry houses. This technology can be a supplemental source of electricity for lighting and ventilation system. In the study conducted by Cui et al. in 2020, it was said that PV technology could provide 70 percent electricity savings and a reduction of CO_2 emissions of 6.23 tons annually. In 2017, a local poultry farm raising 36,000 heads of chicken per production cycle in Pangasinan was given financial assistance of P2 million by the Department of Science and Technology (DOST) to buy and install 50 solar panels that would generate power for the poultry farm. These solar panels could sustain the power needs of up to 50,000 heads of chicken per production cycle.

On the other hand, solar energy can be converted into thermal energy using solar collector technology to reduce the use of LPG during the brooding stage. The solar collector absorbs direct solar radiation, which is then converted into thermal energy. Thermal energy is stored in sensible heat, latent heat, or a combination of sensible and latent heat. The solar air heater system uses stored energy to pre-heat ventilation air to warm poultry houses (Zhang and Zhu 2022).

High electricity consumption can also be minimized by considering custom-designed poultry houses in terms of their orientation to the sun and the use of buffers like shade trees, shrubs, and ground cover plants. This can somehow control physical factors like temperature, humidity, solar radiation, and air movement, which can minimize the use of electrical fixtures to control indoor conditions. This can also contribute to the alleviation of heat stress which affects the mortality of broiler chickens.

Since poultry houses generate a high volume of manure, it can also be considered a secondary renewable energy source. Bird's manure can be converted into biomass energy by obtaining methane using an anaerobic digester. Using anaerobic digestion technology to produce methane from manure waste can also reduce total N and P from wastewater discharges, minimizing its eutrophication potential. Composting can also be done to minimize eutrophication potential. Composted manure releases nutrients slowly which causes less N and P to enter surface water and groundwater. Composting also eliminates pathogens that can harm broiler chickens' health. Higher mortality rates caused by these pathogens can lead to economic losses. Another method that can prevent animal waste from coming into contact with runoff and water sources is the creation of clean water diversion. This can be done by establishing rain gutters and downspouts, earthen ridges or diversion terraces, and the installation of a catch basin. These can keep precipitation runoff clean by directing precipitation away from the manure.

High water consumption was also observed in broiler production for both types of houses. In order to reduce its dependence on municipal water systems, which will also minimize its water bills, rainwater harvesting can also be considered. Another measure to improve the environmental performance of broiler production should deal with more efficient water quality management, such as wastewater treatment. It was observed that wastewater from the poultry farm was not treated before disposal. On the other hand, adding adsorbents like activated carbon, silica gel, and zeolite to the bedding materials can also reduce the odor emitted by poultry manure.

These technologies can be considered to minimize the environmental impact of broiler production. Since there are only slight differences in the values of the environmental impact potentials, these recommendations can be made to equalize the impacts of both poultry houses further. However, a further study on its cost-effectiveness and efficiency may need to be conducted to know its sustainability.

SUMMARY AND CONCLUSION

A comparative analysis of broiler production between conventional and tunnel-ventilated poultry houses was conducted in General Aguinaldo, Cavite. Life cycle assessment was done to evaluate the environmental performance of both types of poultry houses. The system boundary of the study is the operation involving broilers from brooding to post-brooding. Inventory of the material and energy input and output was conducted through direct measurements, interviews of key informants, and secondary data. The impact analysis was done using the results of the inventory. The environmental impacts include global warming potential, eutrophication potential, and acidification potential. The impact potentials were analyzed based on the functional unit per harvested animal.

The inventory analysis revealed that producing 10,000 broiler chickens in the conventional poultry house required 10,553 kg of feeds, 305.40 kg of LPG, 217.95 liters of diesel, 6,343 liters of water, and 203 kWh of electrical energy. It generated 1,391.02 kg CO_2, 0.1526 kg SO_2, and 0.0096 kg CH_4, 7,916 kg manure, and 176,896 liters of wastewater containing 94.9 mg/L of nitrogen, and 65.3 mg/L of phosphate. On the other hand, the production in the tunnel-ventilated poultry house required 8,614 kg of feeds, 185.09 kg of LPG, 66.94 liters of diesel, 18,406 liters of water, and 1,127 kWh of electrical energy. The process 1,488.57 kg CO_2, .1502 kg SO_2, and 0.0088 kg CH_4, 7,979 kg of manure, and 179,254 liters of wastewater containing 368.65 mg/L of nitrogen and 101.72 mg/L of phosphate.

The inventory analysis showed that broiler production with the tunnel-ventilated house had higher GWP and AP values because of its higher electricity consumption. Higher eutrophication potential in the tunnel-ventilated house is also reported because its wastewater is more concentrated with solid waste, as all of the accumulated manure is not released out of the effluent pipe until the last day of harvest.

In conventional poultry houses, the brooding stage has higher GWP (62.24%) and AP (61.36%) because of the consumption of LPG fuel to heat the air inside the facility. On the other hand, the post-brooding stage in tunnel-ventilated poultry houses still had higher GWP (51.63%) despite the use of LPG during the brooding stage. Higher GWP in the said stage is due high electricity consumption. Similarly, higher AP (56.11%) in the post-brooding stage in the tunnel-ventilated system is also due to its high electricity consumption.

The tunnel-ventilated system was also higher in terms of the equivalent GWP and EP per harvested live chicken. Interestingly, it can be noticed that conventional production had higher AP per harvested live chicken because of the higher amount of LPG fuel consumed per bird during the brooding period using such type of ventilation. However, there is very little difference between the equivalent GWP, EP, and AP per harvested live chicken between the two types of poultry houses. It must be noted that these values can only be applied to the specific design of poultry houses and management practices mentioned in the study with consideration of the climatic condition of the Philippines.

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VERTICAL COORDINATION IN INDONESIAN DAIRY INDUSTRY: A COMPARISON OF PERFORMANCE ON MILK QUALITY OF TWO REGIONS

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ABSTRACT

The alterations in the institutional environment within the Indonesian dairy sector have had an impacted the industry's strategies regarding their partnership with dairy cooperatives and farmers. A previous study found that rising consumer milk quality standards drive vertical coordination, in which the industry is more closely aligned with producers (farmers). The study attempts to answer why some regions have better milk quality than others. This study compares two regions in Indonesia which have a different performance in producing milk quality. This study's research methodology consisted of in-depth interviews with 29 key informants in the period of 2013 and updated in 2018, supplemented by secondary data. Two regions, namely East Java and West Java, were chosen as the focus of this study. The study demonstrates notable disparities in the implementation of vertical coordination within the milk value chain between the two areas. Vertical coordination is strongly influenced by the power of the lead firm and the regional characteristics in each region. Furthermore, vertical coordination positively improves the quality of fresh milk in both regions, with East Java having the best milk quality. Improving the performance and sustainability of the dairy value chain requires consideration of institutional arrangements, particularly those related to contract transparency.

Key words: institutional environment, institutional arrangement, partnership, total plate count

INTRODUCTION

Dairy is the biggest revenue producer and fastest growing category in the food and beverage industry, not only in the high-consuming mature markets, but also in emerging markets, in Asia particularly. Indonesia is such a market, where dairy production and consumption grow faster than GDP. The Indonesian dairy sector had been highly regulated, especially in the period of 1980-1997, in contrast with trade and investment policy reforms elsewhere in the economy. The regulations, aimed mainly at fostering the development of the local industry, included an import ratio requirement, import tariffs, an import licensing scheme, and restrictions on investment in milk processing (Erwidodo and Trewin 1996). These policies have resulted in a significant increase in the number of dairy farmers and dairy cattle, in fresh milk production and in other end products, but they imposed substantial costs on consumers and the economy (Erwidodo and Trewin 1996; Riethmuller et al. 1999). However, since the 1997/1999 economic crisis, the Government of Indonesia released a policy reform loosening the regulations under the pressure of the IMF.

The policy reforms brought market liberalization and had an impact on all the stakeholders involved in the dairy industry. With the relaxation of import controls and the removal of the obligation for processors to purchase domestically produced milk, the pressure is obviously high for the Indonesian dairy industry to dramatically improve its productivity level (Beghin 2006). The strong intervention of government in the past created a high dependence of farmers and cooperatives on the industry, and it makes them not always ready to face the dynamic market. The outcome was that many dairy farmers faced serious constraints in accessing essential inputs and in selling their products.

The policy reforms also affected the ability of dairy producers (mostly smallholders) to meet the high-quality standards. There are differences in (private and public) food quality standards. The consumer trend is to be more demanding on specific quality attributes and consumers are much more aware about food safety issues (Dries and Swinnen 2004; Swinnen 2007). This drives the industries to procure the dairy products with a high-quality standard, but farmers are having difficulty in fulfilling the required milk standard. As a result, there is felt need for vertical coordination in the value chain (Bijman et al. 2011).

In a situation where the government's role in supporting dairy development is decreased, the private industries began contracting farms and rural households to provide basic inputs in return for guaranteed and quality supplies. The World Bank indicates that in the absence of appropriate public institutions, private contractual initiatives, particularly from large food and agribusiness companies, are emerging to overcome the obstacles (Van Berkum 2007). This is also supported by Swinnen and Maertens (2007) who argued that liberalization caused disruption of government control in agricultural institutions and is followed by the emergence of new forms of vertical coordination. New forms of vertical coordination are no longer state-controlled but are introduced by private companies which they called private vertical coordination (Swinnen and Maertens 2007).

This study examines the case of the Indonesian dairy industry and shows how the institutional arrangements influence the performance of the industry. In this study, an institutional arrangement is defined as a set of rules or agreements governing the activities of a specific group of people pursuing an objective. The institutional arrangement is also termed as "governance structure" and can be thought of as vertical coordination which is varying between the two extremes of spot market exchanges (0) and full ownership integration (1). Vertical coordination refers to the coordination and integration of different stages in a supply chain, specifically from raw material production to final product distribution. Several studies have examined vertical coordination in the context of the dairy industry (Dries and Swinnen 2004; Dries et al 2009; Szabó and Popovics 2009; Falkowsky 2012; Saenger et al 2013; Kilelu et al 2017a; Kilelu et al 2017b; Zhong et al 2018; Hayer et al 2019; Ekumankama et al. 2020). One study by Dries et al (2009) analyzed the use of contracts in European transition economies to facilitate vertical coordination in agri-food supply chains, with a focus on the dairy industry. This study found that contracts were increasingly being used in the dairy industry to enhance coordination between producers, processors, and other actors in the supply chain. In a separate study, Dries and Swinnen (2004) examined how foreign investment affected the enforcement of contracts to promote suppliers (farmers) to meet the necessary standards and facilitate vertical coordination. The processor offers assistance programs aimed at enhancing access to technology, credit, and other inputs, as well as improving supplier management. The contracts and assistance programs are strategically coordinated to surmount imperfections within the market.

Previous research examined the implementation of vertical coordination by conducting comparative analyses across multiple countries and assessing its effects on various sectors within each country (Dries et al. 2009). In the meantime, a number of additional studies examined the execution of vertical coordination within a single country, with a specific emphasis on a particular sector, such as dairy. These studies evaluated the effects of such coordination on the performance of this sector (Kilelu et al. 2017a; Kilelu et al. 2017b; Hayer et al. 2019). Numerous prior studies conducted performance evaluations among cases within the same sector that employ vertical coordination strategies (Zhong et al. 2018). Several scholarly studies examined the implementation of vertical coordination in the supply chain, specifically focusing on the performance of suppliers (farmers) (Dries and Swinnen, 2004; Falkowsky, 2012; Saenger et al. 2013). However, further investigation is still necessary to completely understand the specific mechanisms and outcomes of vertical coordination in the dairy industry, especially when contrasting the two areas. The study looked at the dairy industry performance only from the angle of milk quality which is produced by farmers or cooperatives. The study attempted to answer why some regions have better milk quality than others. This study compared two regions in Indonesia which have a different performance in producing milk quality. Are the differences in quality between the two regions influenced by differences in institutional arrangements? Two regions were selected in Java, East Java and West Java, where East Java has better quality of milk compared to West Java. At the national level, the achievement of national milk quality standards (SNI) reached only 12 percent on average, while the achievement of SNI in East Java reached 75 percent (Morey 2011).

The milk quality differences between these two regions are interesting to study: it can be an entry point to examine dairy development from a regional perspective, and also from a perspective of different institutional arrangements. The differences in performance of the dairy industry between West Java and East Java can be influenced by the institutional settings, which might have an impact on the way actors are implementing their plans in the industry. It leads to the following sub-questions:

1. How is the improvement of milk quality organized in East and West Java?
2. How does partnership between lead firms and other actors in the value chain in each region affect the improvement in milk quality?

Overview of the dairy industry in Indonesia. In the last ten years, Indonesia's dairy industry and dairy products have experienced a high increase in demand with a rise of over 10% on an annual basis. The increase is due to the changing consumer habits and population growth, and the increase of incomes (Beghin 2006; Morey 2011). Traditionally, dairy consumption, especially of fresh milk is not part of the Asian diet, particularly not in Indonesia (Dong 2005). Dairy products mostly are consumed as powdered milk and as sweet condensed milk. In 2020 per capita domestic milk consumption is still considered low (16.27 kg/capita/year) which is significantly less than other Southeast Asian Countries, e.g., the 36.20 kg/capita/year in the Malaysia, Myanmar (26.7 kg/capita/year and the 22.2 kg/capita/year in Thailand (Daryanto et al 2021). However, Tetra Pak (2014) reported that milk consumption in Indonesia has the highest growth rate in ASEAN countries at 4.8% per year over the period 2006-2010.

About 77% of the Indonesia's current milk demand is fulfilled by imported milk and milk products, amounting to US$ 1.1 billion per year, while the rest comes from domestic supplies. Indonesia meets its domestic consumption through annual imports amounting to 3.37million Metric Ton (MT) of (whole and skimmed) milk powder and condensed milk, mainly from Australia and New Zealand, and also from the EU and the US, which is equivalent to 77% of the total domestic consumption. The country's demand for milk in 2021 stood at 4.31 million MT with local producers supplying approximately 935,000 MT (USDA FAS 2021).

The domestic milk is supplied by approximately 100,000 small farmers who are members of their local dairy cooperative. Based on data from Statistics Indonesia (2021), the small farmers hold approximately 557,000 dairy cattle, producing 935,000 MT which are mainly concentrated in West, Central and East Java (97% in Java and with a small proportion of around 3% in Sumatra and Sulawesi). Farmers typically have 3-4 cows, while only 1% of the farmers have a herd size between 50 and 100 cows. The average daily milk production is 11.5 L/head with an average lactation period of 271 days thus, the average production is about 3,139 L per lactation. This last figure has increased on an annual basis over the past 5 years for members of dairy cooperatives at 42% (in total), however the average is brought down by individual producers where productivity has grown by only 19% (Morey 2011; Moran and Morey 2015).

The dairy processing industries (DPIs) in Indonesia comprise of major local companies (e.g., Indolakto and Ultra Jaya) and multinational companies (e.g., Frisian Flag, and Nestlé). Recently, several multinational companies like Fonterra (New Zealand), Arla (Denmark), Mitsui (Japan) and Greenfield (Australia) entered the market. There are about 40 DPIs in Indonesia, in which six large firms account for 85 percent of domestic milk processing. West Java has the six largest DPIs from the more than 30 DPIs located in this region (Morey 2011). This large number of DPIs shows that the dairy market situation in this region is more crowded compared to other regions. Frisian Flag is located in this region and is key international player who produces 280 MET of liquid and condensed milk per year. Danone Dairy (until 2014 when it sold its dairy business to Indofood), which produces infant nutrition, and Unilever which produces frozen dairy products such as ice cream, were also located in this region. In addition, West Java also has local players such as Indolakto, which is part of the conglomerate Indofood CBP Sukses Makmur, and is one of the largest dairy brands in Indonesia with a market dominance in UHT and sterilized milk. The other key local player is Ultra Jaya, which has joint venture 3,000 cows of dairy farm in Bandung. Many DPIs established their plants in this region because of the proximity of consumers. West Java is one of the regions with the largest population in Indonesia with Jakarta, the capital city.

In East Java, Nestlé is the key international player in the industry in the liquid and powdered dairy product sector and it produces 116,000 MET of milk per year. Another DPI in East Java is Indolakto, which has also been operating in this region for many years. The company focuses on milk processing by using powdered milk as well as raw material. This explains why it does not require so much fresh milk compared to Nestlé. In 2012, Indolakto has also established a new milk processing plant in the district of Pasuruan, with a capacity of 1,000 tons per day.

Analytical framework. The theoretical framework designed to address the key research questions of this study draws on value chain analysis, local-regional theories, and concepts and concentrates itself on a regional level. It is composed of two main elements: first, value chain factors and second, regional characteristics. In this analysis, we emphasize those value chain factors related to governance that many scholars also relate to governance and coordination (Williamson 1985; Altenburg 2006). In this framework, we tried to connect the value chain analysis with new institutional economics analysis while mapping the regional development analysis with macro and micro levels.

It is important to note that Institutional Economics especially the New Institutional Economics (NIE) operates at two levels, macro and micro. The macro levels deal with the institutional environment, or the rules of the game, which affect the behavior and performance of economic actors and in which organizational forms and transactions are embedded. Williamson (1985) describes it as the set of fundamental political, social, and legal ground rules that establish the basis for production, exchange, and distribution. Rules governing elections, property rights, and the rights of contracts are examples of ground rules that make up the economic environment.

The micro level analysis, on the other hand, also known as "institutional arrangements", addresses arrangements between economic units that govern the ways in which these units can cooperate or compete. We can define the institutional arrangements as a governance structure which includes vertical coordination as a mechanism within a value chain analysis. Vertical coordination, on the basis of this, refers to how products move through the supply chain from production to consumption (Hobbs and Young, 2000). Vertical coordination, in its broadest sense, describes the synchronization of the various steps in the vertical marketing channel from producers to consumers in order to address supply and quality issues (Götz et al. 2009). Spot markets are not encompassed within this framework, as they solely rely on price agreements for the exchange of commodities. The concept encompasses both Production partnerships and vertical integration (Swinnen and Maertens 2007). There exists an accepted view that placing a higher priority on the quality and/or quantity of raw materials results in a more significant shift from spot market transactions to more sophisticated vertical coordination mechanisms. Vertical coordination is viewed as a continuum, implying that the more interdependent the activities of the seller and buyer, the more coordination is required (Zhong et al. 2018). A productive partnership can be characterized as a form of vertical coordination strategy that involves collaboration between independent firms. In this specific mode of collaboration, the participating firms engage in a reciprocal sharing of common interests, as well as knowledge and resources, with the aim of enhancing the overall efficiency of the value chain (Götz et al. 2009).We assume that the institutional environment affects the actors in the value chain also to choose specific governance structures. The institutional environment c an create differences between regions and it might bring differences in the setting of institutional arrangements in a specific region. This could explain theoretically why actors choose different governance structures or different institutional arrangements. For example, many scholars agree that vertical coordination or hybrid forms of governance that are closely related to vertical integration are most suitable for improving the socio-economic performance of producers, thus reducing the gaps between the local market and the export market and enhancing the overall performance of the supply chain (Arias 2007).

Figure 1 explains how changes in the institutional environment in any period can be followed by a change in institutional arrangements. In the case of the Indonesian dairy industry the changes in the institutional environment occurred through the changes in government policy which previously very strongly regulated the sector and now liberalized it. It means that the industries had no control anymore on their imports, no obligation to purchase locally produced milk and that it was easier for foreign investment to come to Indonesia. These changes were responded by the units/actors in the dairy industry with changes in their institutional arrangements. However, those changes in the institutional arrangements may differ between regions which of course will result in differences in outcomes across the regions.

Theoretically the reason for an actor or unit to choose or change their institutional arrangements is motivated by the need to reduce or minimize the transaction costs. For instance, selecting cooperatives can be an alternative for governance to reduce transaction costs. As stated by Iliopoulos and Cook (1999, 78) cooperatives "represent a hybrid organizational mode blending market forces with elements of internal organization designed to minimize transaction costs". In addition, the contractual arrangement can also be an alternative for reducing transaction costs. The alternative institutional arrangements have developed to minimize the transaction costs of ownership and contractual arrangements (Eaton et al. 2008).

Dairy products have a specific characteristic of being perishable and having a high asset specificity which potentially affects the *holdup* problem in the transactions. Many of the benefits farmers receive from establishing cooperatives originate from the holdup problem and the opportunistic behavior associated with asset fixity (Staadz 1994). Royer (1999) gave an example related to these problems. In order to force the farmers to accept lower prices, the processor can refuse to accept the delivered commodity. On the contrary, the processor can also potentially be threatened by farmers who hold up to supply their product (in case there are no other suppliers), when the processor has invested in a specific (idiosyncratic) plant. A strategy for producers to eliminate or minimize the holdup problem is for them to purchase the processing plant (i.e., to vertically integrate their operations) this could provide them with the necessary market power and guaranteed market access.

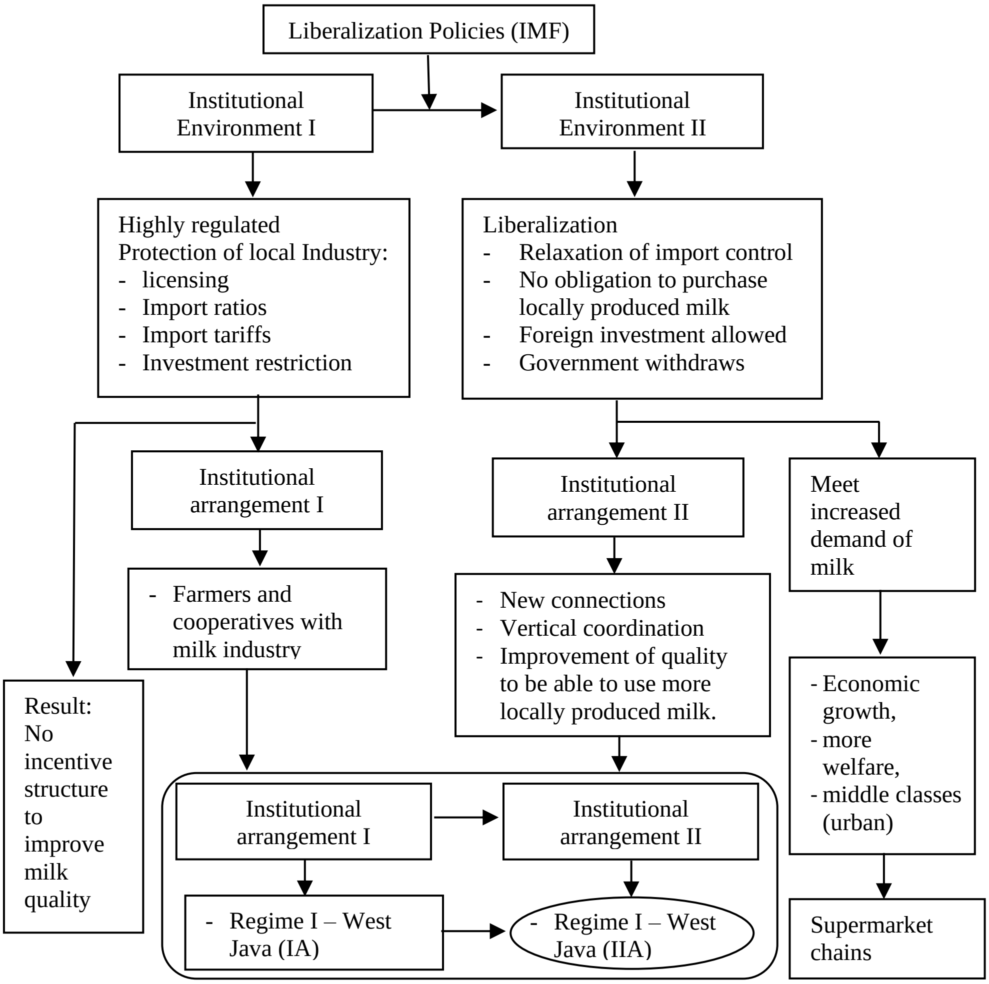


Fig. 1. Analytical framework: The institutional changes in Indonesian dairy industry

RESEARCH METHODS

The research strategy for this study is based on a comparative case study of the value chains of milk production in two different regions, East Java and West Java. The data and information for this article are gathered in two periods: the first condition at 2013 and then updated by the condition of 2018. These two regions were chosen because they are the major producers of milk in Indonesia. East Java province contributed 54.2 percent of total national milk production, with West Java contributing 33 percent. Furthermore, the two regions perform differently in terms of the quality of milk produced (Morey 2011).

The methodological design considers the guidelines set by Yin (2004). A case study is an empirical inquiry that researches a contemporary phenomenon within a real-life context, especially when the limits between the phenomenon and the context are not evident. It benefits from a previous development of theoretical propositions to guide the collection and analysis of data. The construction of the cases integrates the following elements: the regional characteristics, the institutional environment, the regional setting of the dairy value chain including institutional arrangements, a description of the value chain in both provinces. The purpose of the comparison is to find out what are the effects of the different regional characteristics and governance configurations of the chain, on a meso-level of intervention, at the regional level.

The research relies on a combination of secondary and primary sources. The secondary sources come from documents from the Ministry of Agriculture, the Union of Indonesian Dairy Cooperatives (UIDC/GKSI), NGOs, universities, and private firms. The primary sources come mostly from key stakeholders (chain operators), government officials at national and district level and experts of the Value Chains (VCs). In-depth interviews using semi-structured questionnaires with key informants of the VCs in different regions where the research took place were also conducted. A total of 29 key informants were interviewed: 16 cooperative's leaders, 3 dairy experts/consultants, 4 government officials and 2 milk collectors. Four cooperatives were selected from Bandung and West Bandung regencies in West Java and four cooperatives from Malang and Pasuruan regencies in East Java. The selection of cooperatives was based on member size and milk production. Both in West Java and East Java, two cooperatives were selected that represent the larger sized cooperatives (between 4,000 and 10,000 members) and two cooperatives that reflect the smaller/medium sized cooperatives (between 100 and 2,000 members). Three dairy processing industries (DPI) in each region were also selected based on the share of milk sales that have been sold by the cooperatives to these industries.

RESULTS AND DISCUSSION

The organization of improving milk quality in West and East Java:

Milk collection. Milking was usually carried out twice a day at 04:00 – 06:00 AM and 02:00-04:00 PM. The milk was collected into milk churns with a capacity of 15 or 25 liters depending upon the production or numbers of lactating cows. The milk was taken to the nearest Milk Collection Center (MCC), usually owned by cooperatives, on foot, by bicycle, or by motorbike. At this point, the milk was transferred into 50-liter churns or in a milk tank, and the quality was screened using an alcohol test and a lactometer by the technical staff of the dairy cooperative. In some cooperatives, mostly in East Java, the staff of cooperatives also took a sample of the milk to test the composition of milk and the bacteria content (total plate count/TPC). This testing was performed in the presence of the farmers' representative, and both parties countersigned for milk quantity and quality.

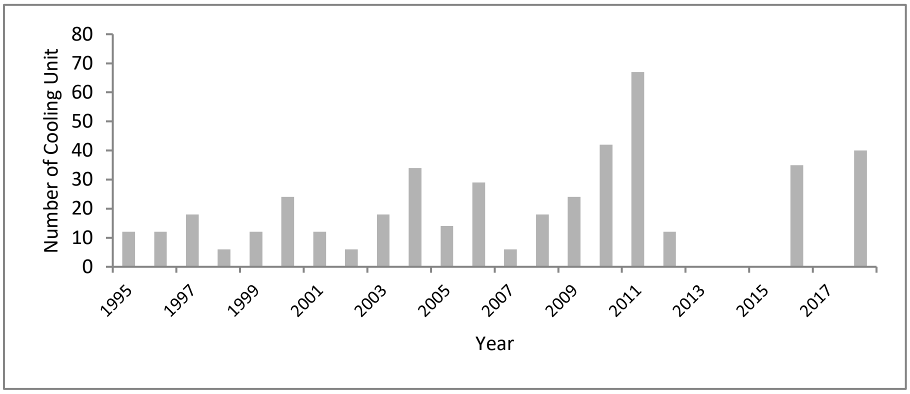
In most of the areas, MCCs were usually the bulking centres for the dairy cooperatives. In every village, there was at least one MMC to which small-scale individual farms from the village deliver their raw (fresh) milk. In small villages, there was often only one MCC, while in medium and large villages, there could be two or more. MCCs were mostly established by cooperatives or by individuals (collectors) who then delivered to the processors[1].

An MCC is managed by cooperatives or in some rare cases, by a collector located in the village. The cooperative arrangements between cooperatives and dairy processors were determined by a written contract (especially in East Java), but verbal agreements between both parties also existed. Cooperatives or milk collectors were responsible for the interactions with farmers, in collecting raw milk in its storages until the milk truck arrived to collect and for taking samples of the delivered milk for quality tests. For their services, cooperatives received a percentage (fee) of the total turnover. The

[1] Side-sales or 'hawking' to the middlemen who buy milk from the farmers and sell the milk to the industry. The collectors become an alternative market for the farmers when cooperatives cannot offer the services as expected by the farmers. Some cooperatives can be inefficient in term of high overhead cost and selling to or being members of the cooperatives might then be less advantage compared to selling to the collectors.

fee varied and depended on the condition of the cooperatives. The MCC's role as an intermediary significantly reduced the transaction costs of the processors. To calculate this fee, an example might be that one of the few large dairy farms receives Rp 6,000 (equal to US$ 0.39) for high quality milk, while a cooperative member in the same region receives Rp 4,700 (equal to US$ 0.31) for the same quality.

In East Java, a major milk processor (Nestlé) has been providing milk cooling units (with a capacity of 2.5 tons each) to MCCs owned by cooperatives. In the period 1995 to 2018, Nestlé supported the cooperatives to install in total 441 cooling units (Fig. 2). In addition, the company provided cooling units intensively in the periods 2003 to 2011 which was in line with the quality improvement programs Nestlé developed. In implementing this program, Nestlé signed contracts with the cooperatives, requiring both parties to implement a "standard operating procedure" (SOP) in handling and managing fresh milk from the farmers. The SOP, for example, points out that MCC's staff must send the milk at the MCC to the processor when it has cooled down to four degrees.



Source : Nestlé, 2013 and update it in 2018

Fig. 2. Cooling units installed in MCCs by Nestlé in East Java[2]

There are 22 dairy cooperatives in West Java, of which 80 percent have cooling units at cooperatives level. Mostly the cooperatives worked with the milk processor to install a cooling unit. Most of the MCCs however, did not have cooling units; only about 20 percent have a cooling unit (GKSI 2012). For example, KPSBU Lembang, the biggest dairy cooperative in West Java, only has five cooling units in 100 MCCs which supply milk to the cooperative. The cooperative used milk trucks to collect milk from the farmers which were not equipped with a cooler. The trucks sent the milk to the cooperative, and then it was cooled down to below 8-degree Celsius before it was carried to the dairy processing plant. If the milk delivered had a temperature of more than eight degrees, the company will give a penalty of Rp. 50 per liter (equal to US$ 0.003).

Some cooperatives in West Java received a grant from the central government to buy a cooling unit. The grant is allocated by the local government to some cooperatives in this region. However, the grant is not enough to buy a cooling unit because it is rather small. The regional government installed cooling units only in three MCCs in 2010, and four units in 2011. The limited amount of the grant might be a symbol for the limited attention to the dairy sector by the local government. Frisian Flag from 2004

[2] In 2013-2015 and 2017 there were no cooling units being installed by Nestlé due to a lot of goverment assistance in installing the cooling in some MCCs in dairy cooperatives

onwards also started providing cooling units, financed partly by the aid envelope of the Government of the Netherlands.

Support for dairy farms in milk quality improvement

West Java[3]. There are several programs initiated by the dairy processing industries (DPI) to support the farmers and cooperatives. The supporting programs were mostly conducted through partnerships with cooperatives. In their programs, the DPI did not directly support farmers individually, out of the consideration of cost efficiency (high transactions cost). However, not all cooperatives can be a good partner due to the differences in both visions, the organisation's objectives, and their organisational structure. This made the DPIs more selective in selecting their partners and apply certain requirements to obtain the technical assistance (TA) and financial assistance (FA) from them.

In its partnership programs, Frisian Flag Indonesia (FFI) provided loans (with small or no interest rate), grants, and assistance to the cooperatives for the procurement of equipment required to maintain the quality of dairy production and productivity as well. As a part of its social commitment, FFI presently assisted eight dairy cooperatives in West Java. In its approach, FFI preferred to cooperate with dairy cooperatives that share a similar vision as the company, thus the cooperatives would strive to improve dairy farm productivity and milk quality by learning together with FFI. Therefore, FFI implemented various partnership programs whose goals were to improve the process of milk production and milk procurement undertaken by farmers and cooperatives. The company provided technical assistance such as training cooperatives' extension workers, assistance in setting up a laboratory and handling of milk, laboratory equipment installation, developing systems and procedures, setting up logistic systems and recommending bonus and penalty systems.

Ultra Jaya, the domestic-owned company, previously had partnership programs with the largest dairy cooperative in West Java, namely KPBS (Koperasi Peternak Bandung Selatan). In 1979, the company provided a loan to establish a milk treatment plant (MT) in the cooperatives; a loan which is repaid by deduction of milk payments. The milk treatment became a milestone in developing the dairy industry in West Java since milk spoilage was high at that time and the farmers could not send the milk to the dairy processor daily. Currently, this company has applied a different approach in implementing partnership programs by establishing a modern dairy farm which has an area reaching almost 60 hectares located in Pangalengan, south of Bandung, West Java, which was already a site for dairy farming in colonial times. The dairy farm is a joint venture with KPBS in which the cooperative has a share of 25% of the capital. The farm has 2,600 cows and selected 75 farmer members of the cooperative who learned to practice dairy farming and herd management. Each farmer managed 15 cows and stayed in a house on the farm for 12 months. When this program finished, the farmer was expected to be able to practice good farming practice on their farm. Ultrajaya only supported cooperatives in West Java and it covered only four cooperatives with which it has a long relationship.

Indolakto had also some partnership approaches with cooperatives and farmers. The company supported cooperatives by providing a cooling unit, credit for cattle, and a low price for raw materials for feeding (e.g., wheat pollard). To support the farmers, the company has field officers called "KUD services" with the aim to introduce good dairy farming practices (GDFP). However, the program is still limited in its reach to farmers in West Java. The team's effectiveness is strongly related to the coordination between farmers and the cooperatives since most of farmers are controlled by dairy cooperatives.

[3] This and the following section are based on a series of interviews foreign owned and Indonesian owned DPIs and the cooperatives' board in both regions.

East Java. Nestlé applied the principle of social responsibility in cooperating with the dairy farmers through the dairy cooperatives (Nestlé 2013). Nestlé's partnership in East Java, involved 41 cooperatives and farms, 40,000 dairy farmers with around 65,000 to 70,000 cows (Scharer 2014). The partnership programs aimed to ensure long-term absorption of milk capacity in the region. In addition, Nestlé also supported suppliers in establishing/improving their operational set-up; accessing milk collection equipment (e.g., transport and cooling units) and provided training for cooperative employees. Nestlé provided technical and financial assistance in order to improve the milk supply chain (milk collection and procurement activities) and to strengthen farmers' viability (milk quality, feed, fodder, cement, animal health, and biogas). By implementing the program through a written contract, Nestlé forced the dairy cooperatives to provide milk to the MCC at a required standard, to ensure the cleanliness and hygiene quality of the purchased milk. Currently, there have been more than 430 MCCs built in most of the cooperatives in East Java. Most of them are equipped with a cooling unit, electricity generator, and hot water installation, in accordance with the standards required by Nestlé. The company implemented certain programs to support government programs. For example, the company assures certainty of milk procurement as a guarantee for credit repayment to back up the government credit scheme to the farmers.

Nestlé also implemented corporate social responsibility (CSR) programs by building more than 8,000 units of biogas installations. The program is in cooperation with dairy cooperatives, local and foreign NGOs[4] in which it provided the initial funds. Nestlé provided the soft loans (with no interest) to build the biogas installations in the farmers' houses, supplying the energy (biogas) for cooking and lighting. The farmers got the credit from the company through their cooperatives. The company supplies the credit fund through the cooperatives based on the number of members in this program. The credit has a maturity of one to three years payment of which is deducted directly from the sales of fresh milk to the cooperative. Some cooperatives involved in this program provide special resources, such as expert labour to build the biogas installation. In East Java, more than 75% of Nestlé suppliers are participating in this program.

Greenfield, the other DPI in East Java, has its partnership programs by providing five units of Milk Collection Centres with an installed capacity of 25 tons/day and supporting individual farmers directly, with no relations with dairy cooperatives. The company used the MCC as an instrument for coordination with farmers and between the company and farmers. In each MCC, the company encourages farmers to establish farmers groups. The company also has a team of agri-service officers who regularly visit the farmers. To fulfil the needs of farmers, Greenfield provided some farm inputs like milk cans, drugs, vaccines, cement, and high-quality feed that farmers can purchase and pay by deducting the credit from the fresh milk payments. The company distributed the farm inputs through the MCC. In addition, Greenfield has provided veterinarians to manage animal diseases and to assist farmers. They stand ready 24 hours a day.

Currently, Greenfield facilitates farmers access to credit from banks with two different schemes, namely: (1) a CSR-individual loan of Rp 30 million[5]/partner with a flat interest rate of

[4] The program is called The Indonesian Domestic Biogas Program (IBDP) or known as BIRU program. The program is implemented by Hivos, working closely with the Indonesian Ministry of Energy and Mineral Resources and SNV Netherlands Development Organization. The first phase (2009-2013) was funded by the Dutch embassy in Indonesia (CCPHI 2012).

[5] Equal to US$ 2000

6%/year and a maturity of 36 months, including a grace period of 4 months; (2) a KKPE[6]-individual loan of Rp 30 million/partner, that is due within 48 months, with a grace period of 4 months. It has an effective interest rate of 4-5%/year. Both credit schemes have the same payment mechanism in which repayment is deducted from the payment of fresh milk collected at the MCC. The milk payment from the company comes as a guarantee to the bank because the farmers have stable incomes from milk sales. To mitigate the risks, the company provides mentoring programs for farmers. The mentoring programs end when the credit is paid off.

In collecting fresh milk, Greenfield applied a computerized system in the MCCs, and it required the farmers to implement the MCC procedures (i.e., SOP). The MCC system has a slogan of "honest and transparent" to express that the system is trustworthy. The milk collection systems have a set of objectives which should transform all the partners including the farmers, namely: (1) to reach good milk and milk quality standards set by the DPI: (2) to have a milk collection process that can be seen directly by the farmers which will cause an increase of trust in the MCC's officers; (3) to pay competitive prices; and (4) to create a modern and efficient MCC.

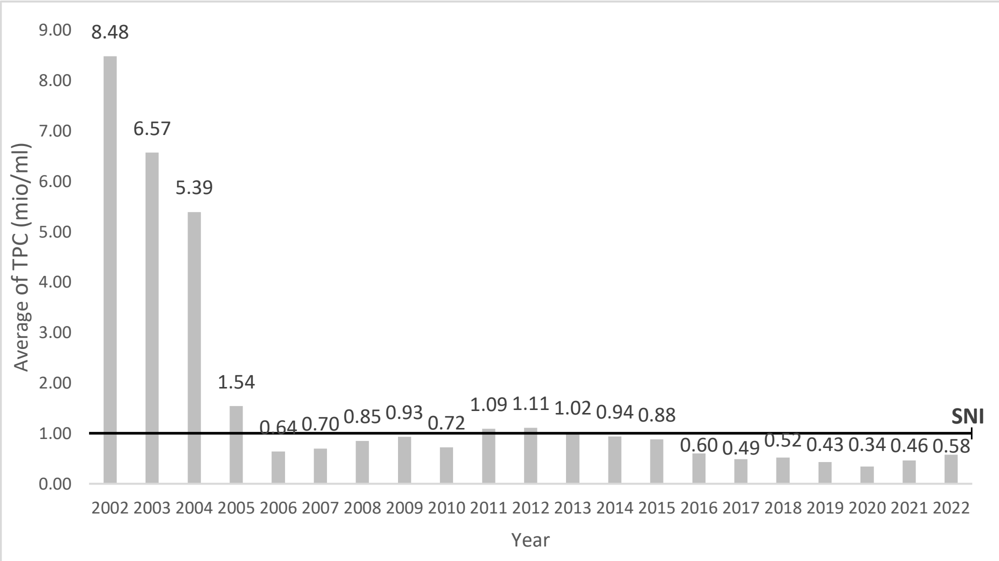
Quality improvement through payment and control system

West Java. DPIs implement milk payment systems (MPS) to improve milk quality. In this system, farmers are required to meet milk quality specifications and are paid incentives or receive penalties accordingly. DPIs paid a higher price for a better quality of milk, and this encouraged farmers to deliver better quality milk to the company. In West Java, the implementation of MPS started in 2001, which was gradually adapted by cooperatives and farmers to meet the milk quality requirements. The milk quality requirements evolved in line with the ability of farmers to fulfil the requirements. Previously, the milk payment was based solely on alcohol and specific gravity tests, and then continued to integrate fat content, fat and total solid (TS) and lastly it considered the amount of protein and bacterial content (TPC/Total Plate Count). However, the implementation of the MPS encountered many obstacles because it is not uniformly applied across the dairy processing industry.

FFI set the price of milk of the farmers or cooperatives based on the quality parameters such as fat content, TS, solid non-fat (SNF), protein and TPC. The milk quality parameters were measured at the collection points by the cooperative and this became the basis for the payment to the farmers. While payments to the cooperative were based on the results of laboratory tests in the FFI plant, FFI used TPC content as a basis for establishing a penalty if it exceeded the standards set. Reducing the number of TPC has become the focus of the FFI partnership programs. FFI supported cooperatives to upgrade the collection process and provided the infrastructure to achieve a high quality of milk. The company provided partnership programs such as to install laboratory equipment, training for laboratory operation and training for extension workers.

FFI also established systems and procedures that are implemented in some cooperatives (about 12 cooperatives that are considered capable and have a shared vision with FFI). Among the 12 cooperatives, KPSBU (Koperasi Peternak Sapi Bandung Utara) is considered the most successful in the implementation of quality improvement systems initiated by FFI. Since the cooperative started the quality improvement program in 2001, the cooperative was able to reach the target and to reduce TPC from above 8 million bacteria per ml in 2002 to close to below 1 million bacteria per ml in 2006 (Fig. 3).

[6] Food and Energy Security Credit, hereinafter referred to as KKP-E, is investment credit and/or working capital provided to support the implementation of the Food Security Program and Vegetable Fuel Raw Material Plant Development Program.



Sources: KPSBU annual report 2002-2023.

Fig. 3. Milk quality improvements at KPSBU cooperative in West Java (based on Total Plate Count/TPC)

One of the systems initiated by FFI is the formation of "price groups" in the dairy cooperative. These groups had a small number of farmers (5-15 people) which are formed by a cooperative as a basis to take milk sampling tests at the MCC. The milk test result determined the milk price and reduced difficulties if milk quality is tested individually. Each member of the group will receive the same price based on the quality grade. The "price group" is an instrument of social control among the members of the group, and they can support their partners to maintain or improve the milk quality.

FFI obtained support from the Dutch government for the implementation of the program mentioned above. In 2001 for instance, the Dutch government allocated not less than US$ 0,88 million to support the dairy development program in the country and ten years later it paid 40% of a US$ 14,3 million project for the development of sustainable dairy villages in East Java. One of the important components of the program is capacity building at the cooperative level where the dairy cooperatives sent 30-40 dairy farmers to be trained intensively as trainers (TOT). From 2003 to 2005 the project continued with a shift in strategy, targeting the institutional building/reformation of the cooperatives (meso-level approach) and had successfully accomplished the objectives of the project. In assisting the dairy cooperatives, FFI followed the cooperative policy of the Indonesian government which assigned to the cooperatives the main role of improving the milk production. Hence, there is no direct intervention at farmer level, but rather advice and recommendations are given to the cooperatives. At present, FFI still continues the dairy development program in collaboration with Indonesian public enterprise and the dairy cooperatives.

PT FFI in collaboration with PT Perkebunan Nusantara VIII (PTPN VIII) and Koperasi Peternak Sapi Bandung (KPSBU) Lembang officially signed a memorandum of understanding (MoU) for the dairy village program as a contribution to the Indonesian government's plan in reaching national self-sufficiency for fresh milk in November 2013. The partnership program for the period 2013-2017 had a total funding of € 10 million (USD 13 million), of which 4 million Euros (US$ 5.2 million) came from the Dutch government, and 6 million Euros (US$ 7.8 million) were given by FFI and cooperatives.

The program aimed to improve the welfare of more than 10 thousand dairy farmers in Pangalengan and Lembang in Bandung district, West Java.

In addition, Ultra Jaya and Indolakto applied a milk payment system like that of the FFI, which used fat, SNF, total solids, protein and TPC as a base parameter for price determination. However, based on interviews with the cooperatives, it was revealed that both Ultra Jaya and Indolakto required lower quality standards than those of FFI, especially for TPC content. FFI set the standard of TPC at a maximum of 1 million per ml of milk, and applied penalties if the milk supplied was above the standard. Indolakto however gave penalties if the TPC content of the milk supplied was above 5 million per ml.

East Java. As the major dairy processing industry in East Java, Nestlé plays an important role in improving milk quality in the region. The partnership programs conducted by Nestlé have a strong influence to set institutional arrangements for quality improvement. By applying a written marketing contract, Nestlé can drive the cooperative and farmers to follow the industry. The agreement is largely aimed at improving milk quality; although it also includes other objectives such as increasing the productivity of dairy cattle. In Nestlé's contract, it is clearly states that each party must fulfill the obligation of improving the quality of milk. The operating instructions of the contract are set in Standard Operating Procedures (SOP), issued by Nestlé. Violation of SOPs implies a reduction of rebates or bonuses received from the milk transactions. The SOPs aim to provide an operational guideline that helps cooperatives fulfill the requirements. In Nestlé's SOPs, the minimum requirements must be applied by a cooperative to be consistent with the fresh milk-marketing contract that has been signed.

In implementing the supporting programs, Nestlé established the MPDD program (Milk Procurement and Dairy Development) which aimed to improve the quality of milk produced and to enhance the operational capabilities of suppliers. Moreover, it attempted to develop trust of Nestlé's suppliers for a long-term sustainable business relationship (Schärer 2014). In this regard, Nestlé set TPC content and TS as a quality parameter on fresh milk on which the payment is based. Some of the steps to take include: (1) the presence of clear standards for fresh milk quality, its handling procedures and requirements for milk storage activities; (2) the "gap assessment" and action plan for the improvement of facilities in order to meet the long-term requirements; (3) the standard fulfillment that is agreed upon and set forth in the fresh milk's sale and purchase contract; (4) the control through MPDD's regular audits; and (5) the agreed upon penalty when the standard is not fulfilled.

Nestlé applied the minimum requirements in the milk marketing contracts. This program started in 2004 by PT Nestlé Indonesia. The minimum requirements that must be implemented by Nestlé's suppliers have some critical points: e.g., at the milk collection point, milk transportation and with the dairy farmers. With the help from MPDD, Nestlé conducted routine audits, controls, and regular milk testing to guarantee food safety. The audit is mandatory for all fresh milk suppliers to ensure that all procedures are in accordance with the standards and conditions set by Nestlé. The audits are divided into two parts, namely a full audit on to the whole operation with each supplier conducted annually, and a partial audit that is conducted monthly. The frequency of the audits can be increased depending on supplier compliance, in line with observation and assessment done by a Field Inspector/MPDD.

At the cooperative level, the minimum requirements are used to ensure that the contract is implemented in accordance with the SOP. The SOP requires that the cooperative provides the MCC with a strict procedure in terms of treating both milk cleaning equipment and hot water facilities. Nestlé also requires trucks for transporting milk to be facilitated CIP (Clean at Place). All equipment must be removed after being used, cleaned and dried.

Nestlé also applies minimum requirements at the farm level. Farmers as members of a cooperative must comply with some procedures, such as a good milking technique in which they must clean the udders before milking and use an antiseptic on the udder after milking. Farmers are also required to clean the milk can and must send milk to MCC using a milk can made from stainless steel or aluminum. The maximum milking intervals are 10-14 hours. If the intervals are too long, it may cause problems with dairy cows and influence the increase in bacterial content. The cooperative used MBRT (Methylene Blue Reductase Test) to monitor the number of bacteria in fresh milk sold by farmers. All the procedures required by the industry serve as a parameter in the audit held by MPDD. If farmers do not comply with the procedures, the cooperative will incur a penalty from the industry.

The marketing contract between Nestlé and the cooperatives also includes a price agreement. In this case, Nestlé applies a price table system based on milk quality. This price table is a combination of the number of bacteria content (TPC) and total solid (TS). The milk pricing based on TPC, divided into 4 grades from a grade 4 (with more than 3 million bacteria/ml) to grade 1 (with less than 1 million bacteria/ml). The total solid in the price table has a range between 11.5 - 12.6 percent. If the TPC reaches grade 1 with a TS above 12%, the cooperative will receive a higher price and this applies vice versa. However, it does not include other incentives, such as competitiveness development incentives,[7] volume growth incentives and feed bonuses. Nestlé also applied transport incentives calculated based on the destination and the volume of milk.. In East Java, the price setting is evaluated every year. The DPI (Nestlé) organized the meeting with primary cooperatives to discuss the development of the dairy industry in the current year and set a target for the next year. In that meeting, Nestlé has the power to deduct price (incentives) for the cooperatives that cannot meet their target.

Institutional arrangements and how they are related with milk quality improvement. The cooperations between DPIs and cooperatives have been implemented for a long time. It started when the cooperatives became an intermediary institution for marketing milk produced by farmers. This was mainly caused by the insistence of the government through a policy of the import ratio/BUSEP to enforce that DPIs should absorb domestic milk[8]. However, previously the relationship was only limited to receiving and paying the appropriate amount of milk volume. The DPIs at that time did not have a commitment to foster and assist the cooperatives, especially in terms of increased productivity and quality. Besides, the farmers or cooperatives were not required to supply milk with a high-quality standard.

Compared with the early period of dairy industry development, the period when the import ratio policy was applied, a change in the market structure occurred. Hosen (2009) argues that the implementation of the import ratio policy changed the fresh milk market structure from buyer market structure to a negotiated market structure leading to a contract system. Consequently, primary dairy cooperatives and GKSI had monopsony and monopoly power in buying and selling fresh milk respectively. The DPIs had to accept the full amount of domestic milk production. Dairy farmers, primary dairy cooperatives and GKSI had the opportunity to set up vertical integration to compete with milk prices in the world market.

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[7] Competitiveness Development Incentive is an incentive which is based on local and international market competitiveness. It paid per kg of fresh milk and composition (TS%). This incentive is not related to quality parameters (TPC grades)

[8] This is the most complex policy instrument affecting the industry; it is a form of non-tariff barrier in which the government controls the extent of milk imports based on the quantity of domestic milk purchased by milk manufacturers. This mix ratio import was referred to in the decision of three ministers, known as the letter of three ministries which it called SKB = Surat Keputusan Bersama (Remenyi 1986; Erwidodo and Trewin 1996).

In the liberalization era, although DPIs no longer have an obligation to buy milk from cooperatives or farmers, the cooperation between DPIs and cooperatives still exists and is still of importance and even deepened. The DPIs still need a continuous supply of fresh milk from local suppliers to secure their raw material for milk processing, since consumers (city's middle classes) prefer fresh milk[9] and want high-quality milk. Thus, the liquid milk processing needs fresh milk as a raw material.[10] The changes in the global market also influence the decision of DPIs to stay in cooperation with milk domestic suppliers. As an illustration, in 2008 the world milk market price was increasing and strongly influenced the milk import price. Consequently, the DPIs decided to source more milk from domestic suppliers because it was more competitive than imported milk.[11]

However, in the absence of an obligation to buy milk only from the cooperatives, DPIs have the freedom to cooperate with other actors in maintaining milk supplies such as with milk collectors. This policy change provides an opportunity for milk collectors to start growing. DPIs in this case use the collectors when it is difficult to negotiate with cooperatives or when they want to grab milk supplies from their competitors especially when fresh milk supply is limited. By providing milk handling equipment and teaching a penetration strategy to the collector, DPIs try to seize milk supplies from their competitors. This phenomenon is explained by one of DPI's managers:

> "We buy milk in that region through the milk collector or through the farmer groups. We support the milk collectors. For example, in Y we teach them the strategy to seize the supply of cooperative members X. We provided cooling units and advised the collector to put it in "blank spots" where the distance between the farmers and the cooperatives' milk collection center (MCC) are far enough. I told him (the collector): "Mr… install the cooling unit there and pay 50 rupiah (equal to US$ 0.003) higher than the cooperative and let them (farmers) move to you ...." It is proved, from that region, he (the collector) got around 3,000 liters of fresh milk per day". (Dairy Processor 132)

Currently, most of the DPIs do not have contract directly with dairy farmers since the number of cattle they have is very small. Contracting directly with small farmers would certainly involve high transaction costs. As a result, contracts are mostly made between DPIs and cooperatives, although not all the cooperatives are willing to close a contract. Some contracts are written and are evaluated periodically. However, some partnerships are also conducted without any written contract. The reasons why contracts are not written will be explained more in detail in the next section. Additionally, the relationships and how they evolved will be explained by comparing the two regions.

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[9] Tetra Pak Dairy index report (June 2013) shows the rising number of Indonesian consumers on liquid milk especially flavored milk. There is a high growth in the consumption rate of flavored milk. Tetra Pax forecast the growth about 6.7 % annually (between 2012 and 2015), a growth rate higher than forecast in China, India, Malaysia, Thailand and the Philippines. The high growth of liquid milk indicates the increased of fresh milk demand as raw material.

[10] In 2012, three types of consumer products that continue to dominate the market are the liquid ready-to-drink UHT milk, sweetened condensed milk, and powdered milk, with a total market share of 26 percent, 35 percent, and 39 percent respectively. During the past five years, liquid ready-to-drink UHT milk grew the fastest by 17.39 percent annually, while sweetened condensed milk grew negatively

[11] The milk price volatility that happened in 2008 made the dairy processing industry concerned to secure their domestic fresh milk supply (Nugraha, 2010). In September 2007, world milk prices (1.25 Butter Fat Skim Milk Powder) increased to US$ 5,225 per ton, where it was only US$ 3,100 per ton in January 2007 and about US$ 3,200 per ton in September 2014.

West Java[12] Milk marketing in West Java is for the major part facilitated by GKSI West Java. Nevertheless, in practice the DPIs can close contracts directly with the primary cooperatives and decide on the amount of milk that will be supplied and on the agreed prices. The role of GKSI is to determine and to negotiate a minimum price based on the milk quality standards. The milk transaction between DPIs and cooperatives is facilitated by GKSI and the payments are directly transfered from the DPIs to the GKSI account before it will be distributed to the cooperatives based on their milk sales. In this way most of the transactions are monitored by GKSI West Java. The amount of milk sales becomes the basis for the board of managers to calculate a fee for financing the operations of GKSI.

In this region, most of the cooperatives are unwilling to go into contracts with DPIs, especially the written contracts. Even though there are no written contracts, the relationship between DPIs and cooperatives is ongoing since they were established 20 years ago. DPIs actually have a desire to close a contract with cooperatives. However, cooperatives often do not want to go into contracts for a longer time because they cannot maneuver then, or they do not have the freedom to sell their milk to another party in case of a difference in prices. It is also due to the differences in the type of contract agreed upon in establishing the relationship. DPIs actually desire to have a contract in particular on a quota not a price contract. Meanwhile, the cooperatives would rather prefer a price contract. This was explained by the cooperative leader and DPI's manager who have been interviewed:

> "To be frank, if we are tied by a contract with the DPI, we are afraid that we cannot maneuver when the milk prices received are decreased" (Secondary Dairy Cooperative 211)

> "Actually, the industries wanted to make a contract with us, but we have a different opinion on the type of the contract; this cooperative wanted a price-based contract while the industries wanted a quota-based contract. Because of this disagreement we did not find a solution, so the contract was suspended. In addition, there was a fluctuation of the international milk price, so it would be more beneficial for us if there was no contract. Even though de facto the price is decided by the DPI, they also did not want to have independent lab check. They preferred to do it on their own" (Primary Cooperative 221)

> "Until now, no one wants to conclude a contract. The idea had been proposed for years, ever since I began working at the company's dairy division. But there was never any cooperative that wanted a contract, because most of the dairy cooperatives have a second buyer. I like to joke with them, asking why their milk supplies are smaller than earlier? And they tell that their milk production was decreased .... I can only joke "Oh, you move to another heart", I said" (Dairy Processor 132)

Although no written contracts are signed, transactions are still going on on the basis of trust. Although, it is not easy to manage such a relationship, the DPIs and the cooperatives can maintain these for a long term. In some cases the silent or unwritten contract can be violated and then it is difficult to undertake legal action related to violations. One of the DPIs' managers conveyed how a cooperative in West Java violated these relationships.

> "We have one of the major suppliers in West Java, namely cooperative X located in Sumedang district. Our supplier has a milk cooling unit and other equipment provided by one of our competitors, the DPI Y. The cooperative X however did not sell their milk to the DPI Y although the DPI Y provided them with milk equipment" (Dairy Processor 132)

[12] This and the following section are based on a series of interviews foreign owned and Indonesian owned DPIs and the cooperatives' board in both regions.

The motivation to violate the agreement may not only come from the cooperatives, but also from the DPIs. The absence of a written contract easily motivates the actors to violate the relationship. It needs good communication and maintaining mutual trust between the DPIs and the cooperatives. For instance, cooperatives expect DPIs to be more understanding and pay attention to the difficulties experienced by farmers at the farm level. Commonly, the cooperatives request the DPIs to increase the milk price in line with the increasing costs of milk production. In this case the price becomes the main signal in the negotiations. As stated by one of the cooperative leaders:

> "It is about trust and good communication with the DPI. However, I cannot say it is a good life. Like now, when the number of cows decreases, the milk production also decreases. In this case, I want to receive a higher price of milk from the DPI. I often personified cooperatives and DPI as husband and wife. Why don't we have any legal ties of marriage while in fact we live together?" (Secondary Dairy Cooperative 111)

At present, there is no public inspection authority as a part of the public institutions which govern milk marketing in the Indonesian dairy industry. The absence of regulation might result in market failure in the industry. In addition, this situation can increase the transaction costs due to the asymmetric information and opportunistic behaviour of agents could result from it. For example, there are still many DPIs which acquire milk without any quality standard and without any commitment to Indonesian national standard (SNI). As a result, it is hard to increase the quality of milk in such a downward cycle. For instance, when the cooperative gives a lower price to the farmers due to the more inferior quality of their milk, the farmers sell their milk to collectors whose milk is also accepted by DPIs.

East Java. Nestlé has become the biggest dairy processing industry with a plant capacity of about 1.2 million liters per day. In East Java, Nestlé is now the dominating DPI and has signed written contracts with 41 cooperatives. This company purchases fresh milk from farmers at an amount of around 578 tons per day (the Nestlé's share is about 34% from the total 1,700 ton per day of SSDN[13]). The written contracts have become an instrument for the realization of Nestlé's partnership programs such as Technical Assistance (TA) and Financial Assistance (FA). To secure quality, this DPI established the so-called 'standard operating procedures' (SOPs) to define the technical requirements in milk handling by cooperatives and by dairy farmers. Of upmost importance in the product quality regulation is the determination of the milk price paid to the cooperatives based on the quality parameters of the milk delivered.

Nestlé designed the contract with cooperatives on a year by year basis. After a year, both parties can stop or extend the contract. Cooperatives tend to extend the contract with Nestlé because they have little choice and also feel quite comfortable with the partnership system of Nestlé. This suggests that relationships between processors and farmers (cooperatives) in the East Java dairy sector are rather stable. This phenomenon is explained by some cooperative's leaders who were interviewed:

> "Most of the milk is sold to Nestlé because the company provided us with the price bonus in quality and quantity. We do not prefer to sell our milk to another region such as West Java because the milk payments are often delayed. Nestlé always pays us on time. We have a long-time relationship with the company, since 1979. Although there are ups and downs as well (the cooperative had to stop due to an imbalance of milk deliveries). Nestlé has invested in the cooling units and assisted us in corporate social responsibility activities in the form of biogas credits" (Primary Cooperative 321).

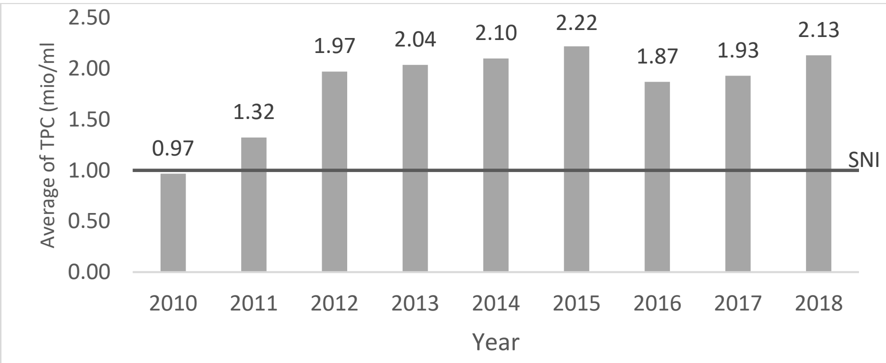
[13] SSDN (Susu Segar Dalam Negeri): Fresh milk which is produced domestically.

"It now looks clear that Nestlé became a dominant player, but when viewed from another perspective, Nestlé actually had a good proposition in terms of coaching and partnerships. But for milk price setting we hope we have a chance to compare it with other DPIs as well. On the other hand, this DPI helps in looking for a cooling unit with higher quality standards". (Primary Cooperative 331)

"In the beginning of the year, Nestlé always invites us to draw up annual programs which become our target on that year. Nestlé not only did transactions with the cooperatives, but also participated in partnership and coaching". (Primary Cooperative 331)

Quality improvement: driving forces and obstacles. Improving quality is a critical factor in dairy industry development in addition to the increase in productivity. Poor quality handling leads to lower milk prices for the farmers. The failures of milk quality handling in Indonesia brought a potential loss/opportunity cost of US$ 4,6 million/month (Stanton et al. 2005). Field observations indicated that most of the farmers still get a lower price because they are not able to meet the quality standards that are targeted. This inability might be due to individual problems of farmers or to collective problems experienced by the cooperatives.

Industries interviewed explained that improving milk quality was the main objective of their farm assistance programs. Although quality improvement programs were implemented it is still difficult in West Java to improve the milk quality standard especially the microbial content (number of TPC). Figure 4 shows that the average number of TPC content in the periods 2010-2018 is still above one million microorganisms per ml of milk, and it is above the maximum required by SNI. In this region, only few cooperatives reached the high-quality standard of milk, for instance KPSBU.

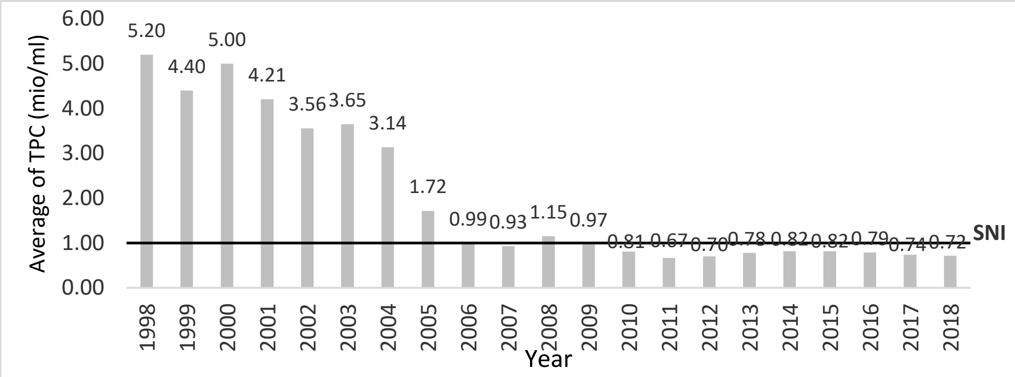


Source: Union of Dairy Cooperatives annual report (2010 – 2022)

Fig. 4. Milk quality improvements in West Java (based on Total Plate Count/TPC)

It is difficult to improve the milk quality standard in West Java because there is still an industry that accepted low quality milk and paid attractive prices. The manufacturers also buy fresh milk from milk collectors in addition to milk collection from the dairy cooperatives. In collecting fresh milk, the collector must compete with the cooperatives, because most of the farmers are members of cooperatives. To attract the farmers, the collectors propose a higher price and apply lower standard of milk quality compared to the cooperatives. As a result, farmers are not motivated to make any investment in quality improvement. Weak infrastructure also caused difficulties in improving milk quality in West Java. Due to the limited funds available, cooperatives could not build adequate infrastructures such as a cooling unit and a milk laboratory. In addition, the DPIs could not provide enough credit for the cooperatives because they have no legal guarantees where both parties have not signed a written contract.

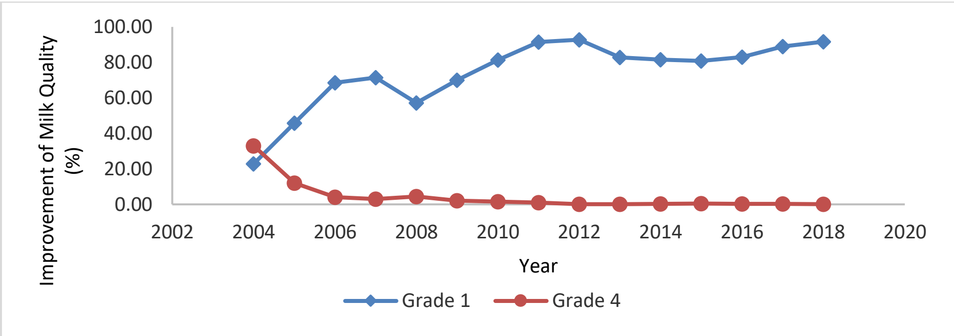
In contrast, the milk quality development in the dairy supply chain in East Java showed positive effects. There are clear indications that the quality of milk produced by farmers in East Java improved recently in terms of TPC (Fig. 5). TPC tend to decrease and since 2006 and it reached below one million bacteria per ml which is higher than the SNI standard.



Source: Nestlé 2013 and update it in 2018

Fig. 5. Milk quality improvements in East Java (based on Total Plate Count/TPC)

Moreover, the partnership program supported by the agri-services (the MPDD) improved milk quality in East Java. When the program started in 2004, most farmers had grade IV and after nine years, it improved to grade I and stayed at that level continuously in recent years (Fig. 6). The milk quality grade is determined on the basis of two parameters; the value of TS and TPC content. It can be assumed that this improvement is a result of significant investment of cooperatives on the basic infrastructure such as a cooling unit, a milk laboratory and standardized MCC and by the training and better management of the farmers. The cooperatives provided the basic infrastructure, assisted by Nestlé in the partnership program. In recent years, by implementing vertical coordination, Nestlé invested considerably in new technology and plant capacity to improve its production efficiency, as well as to improve its production facilities to comply with international and national standards. In addition, the industry applied a feed incentive in the milk price (called feed bonus) received by cooperatives. The incentives were given in the form of raw material used by cooperatives to produce feed concentrate. It stimulates the cooperatives to supply the farmers with good quality feed which potentially result in a high composition of milk especially TS.



Source: Nestlé 2013 and update it in 2018

Fig. 6. The improvement of milk quality (milk grade) in East Java (2004-2018)

Table 1 gives a summary that compares West Java and East Java with respect to enhancing performance in the dairy industry, particularly in fresh milk quality.

Table 1. Comparison of strengths and weaknesses in promoting improved milk quality between West Java and East Java.

Region	Strength	Weakness	Outcome/Recommendations
West Java	- Has Stronger Cooperatives - close to the country's capital (faster and easier access to government assistance)	- More Competition between DPIs - Some farmer development programs (FFI, Ultrajaya, etc.) - Distribution of Milk Collection Centers (MCCs) at low level (difficult to handle milk and potentially causing high bacterial counts) - No Contract or Informal Contract (it is difficult to implement SOPs to improve milk quality without a contract) -	- more challenging to put milk quality improvement programs into action. - Transparency and formal contract implementation require additional attention. - Enhancing the participation of multinational corporations in CSR
East Java	- Less competition between DPIs - Larger farmer development programs - Many Milk Collection Centers (MCCs) distributed (makes it easier to handle milk and prevents an increase in the number of bacteria) - Contract (makes it easier to implement SOPs to improve milk quality without contracts and investment in providing milk infrastructure such as MCC). -	- Medium and smaller cooperatives - far from the country's capital	- Programs to improve milk quality are simpler to implement. - Strengthening and increasing the role of dairy cooperatives

CONCLUSIONS

The Indonesian dairy industry is facing important changes in its milk production and market, but also in government regulations. These changes were driven by the liberalization policies of the Indonesian government and the changes and growth in consumer demand for dairy product. The liberalization resulted in an increasing FDI flow and pushed different stakeholders involved to implement a high milk quality policy. In addition, the milk market changed because the growing middle classes with better incomes wanted more and better fresh milk, which is mostly produced by small local farmers, not in possession of modern dairy technologies. More than other food products, dairy depends on upstream quality, from cow feed to cold chain management to the shop shelves. It thus starts with dairy farming, securing a high quality and safe supply. To procure fresh milk with a higher quality from farmers, national and international companies are competing to get and keep access to these farmers. There are three strategies for the processing industry to secure milk supply from the farmers. The first strategy is to procure milk wherever possible, usually called the 'middleman strategy' where by constructing milk collection centers middle men are trying to get milk also from cooperative farmers. The second strategy is to collect milk wherever possible from cooperatives or cooperative members with payments to cooperative leaders, the 'leaders' strategy'. The Third strategy is to build a sustainable relation with coops via the supply of milk collection centers and (cooling) equipment plus additional services (training, capacity building), leading in principle to transparent contracts, the 'partnership strategy'. A fourth strategy that seems to appear recently and that is not in our analysis, is building a factory together with a large dairy farm, the 'Mitsui strategy'.[15]

In both regions where most milk is produced in Indonesia, East and West Java, milk processors are confronted with both these phenomena, but the situation is different in both regions in terms of industry structure and cooperative behavior. In East Java a monopolistic situation is found with Nestlé as the major DPI. This could have led to exploitative contracts, but Nestlé has an international Corporate Social Responsibility plan which forces it to have transparent relations and open contracts with primary suppliers. The contract makes it possible for Nestlé to finance MCCs for cooperatives which are repaid by deducting from milk payment. Nestlé was also working closely with development cooperation organizations to build biogas installations. So, Nestlé might be the monopolist in East Java, but they are willing to sustain their relations with fresh milk suppliers by means of contracts. This has finally led not so much to a higher production but to high a quality of milk collected.[16]

In West Java there are more players at the supply and the demand side. Formal contracting has not been implemented in this region: cooperatives do not want to close contracts, because they want to offer their milk to the highest bidder and to make profit from price fluctuations. This means that the processing industries when supplying MCCs and training to cooperatives can only control supply through their field managers. This leads to high productivity, because productivity in West Java has always been high from colonial times, but milk quality in West Java has not improved at all.

These situations lead to the fact that we might have had our looking glass at institutional relations but that these could not explain the full situation. There is a need to consider the importance of institutional arrangements, in this case the transparency that arises from contractual relations, vis à vis intermediaries which force companies to another behavior. In Indonesia, milk processors are still

[15] On 21 September, it was announced that Mitsui would go into a joint venture with Raffles Pacific Harvest (the dairy farm) and ABC Kobe (the milk processor) to produce milk in the Bandung area (*Nikkei Asia*, 21 September 2017. Retrieved from: https://asia.nikkei.com/Business/Business-deals/Mitsui-enters-Indonesian-dairy-market-to-milk-growing-demand

[16] In their literature overview of supply chain collaboration Chen et al. (2017) indicate that there is little attention in the literature for social concerns, but also for competition between horizontal collaboration partners.

the dominant player, where elsewhere in Europe or the US it is the supermarket chains (Glover et al. 2014). It is important to note that these phenomena should have their place in institutional economics analysis. Institutional arrangement theory tends to focus on formal relationships, on building trust via contractual obligations. But it is not necessarily so that institutional arrangements, such as contracts, are welcomed by both parties, since relations and thus arrangements might be rather unequal. In that case not only trust and investments in assets, and uncertainty might lead to long-standing relations (Gërdoçi et al. 2017). This might show that bringing Corporate Social Responsibility in the relation could lead to a win-win situation in which both parties value the transparency of clear and open contracts.

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FACTORS INFLUENCING VIETGAP CERTIFIED VEGETABLE PURCHASING INTENTION OF VIETNAMESE CONSUMERS

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ABSTRACT

Since the 1990s, the presence of chemical residues in agricultural products led to severe health problems among Vietnamese consumers. In 2008, the introduction of Vietnamese Good Agricultural Practices (VietGAP) helped consumers gradually become conscious about what they eat. Although VietGAP certified vegetables are more readily available in the market, only a few frequent buyers exist. The emergence of online marketing channels makes it important to target online consumers as samples for the study, which aims to determine the effects of attributes, consumers' attitudes, awareness, knowledge, perceived behavioral control (PBC), and subjective norms on VietGAP vegetable buying intention, using the Theory of Planned Behavior. Data collection was handled in 2022, from 301 consumers in Hanoi and Ho Chi Minh, Viet Nam using an online questionnaire. Principal component analysis and regression analysis revealed that 11 extracted components, income, and vegetable purchasing frequency, have positive effects on the intention to buy, whereas living with family, and age will produce the reverse effect. Vegetable purchasing on an everyday basis has the highest influence, followed by PBC, promotion of domestic products, health concerns, availability, and beliefs. The study also gives some recommendations to enhance the buying intention, such as improvement of production quantity, designs of stores and online interface, production supervision, and promotions of government.

Key words: buying intention, online consumer, safe vegetable, Theory of Planned Behavior, Vietnamese consumer

INTRODUCTION

In Vietnam, as in other developing countries, rapid socioeconomic development is accompanied by agriculture, forestry, and fisheries modernization and industrialization of production. However, along with that development is the problem that pesticide is increasingly utilized in the agricultural field, no matter how much the government tried to enact and expand regulations on pesticide management from 2005 to 2015. Moreover, the available amount of pesticides in the market and the used amount on the farm are strongly connected (Pham et al. 2016). Import of pesticides increased annually from 2009-2012, since 2015, the number has stabilized at around 100,000 tons a year, and farmers have made use of plant protection chemicals as one of their cultivating habits (Nguyen 2020), conveying that there may be prevalent overuse of pesticides in terms of quantity, frequency of application, and time of use. Moreover, among 4,000 permitted pesticides and plant protection substances in Vietnam, only 20% are biological, and the others remain chemicals, which most farmers prefer. As a result, many cases of food poisoning took place all over the country. For instance, from 2019 to 2020, it was recorded as 153 outbreaks with 3,977 injured and 31 deaths (General Statistics Organization 2020). Moreover, the Vietnamese take vegetables as the second most crucial foodstuff, only after rice, however, together with fruits, vegetables use pesticides more than any other crops (Nguyen et al. 2018). This phenomenon raises the importance of food safety and the environment, which affects public health, leading food safety, especially vegetable safety, to be one of the country's most concerned and prioritized social issues when there is an excessive amount of chemicals used to increase the yield on vegetable crops all over the country (Pham et al. 2016). Therefore, in order to cope with the vegetable production issue, the Vietnamese government implemented the "Safe Vegetables" program in 1995 as the premise for the Ministry of Agriculture and Rural Development (MARD) to establish Vietnamese Good Agricultural Practices (VietGAP) in 2008. VietGAP soon became the main standard, and guidelines for producing clean, safe, and health-friendly agriculture products in response to the negative context of food safety.

In recent times, the production of safe vegetables has seen a surge in adopting safety measures such as adhering to global and domestic standards, organic and GAP practices, which has resulted in an increase in the production and cultivated area of vegetables. As of 2018, the number of VietGAP-certified farms rose to almost 1,900, covering an area of 81,500 hectares. In addition, a report by the Agro-Forestry-Fisheries Quality Assurance Department revealed that as of mid-2022, the country certified 463,000 hectares of crops and 6,211 farming establishments as VietGAP certified (An 2022). The percentage of modern retailers has risen from 5% in 2016 to 10-15% in 2019 in the total number of whole retailers in Viet Nam (Hanoi Department of Plant Protection N.A.) with the involvement of more multidisciplinary corporations such as PAN Group, Vingroup, FPT, in the agricultural sector, especially GAP vegetables. In the case of Hanoi, safe vegetable growing models became beneficial to producers and consumers. The city issued many policies to support farmers to promote the production and consumption of safe vegetables (Hanoimoi News 2020). In the long run, the Hanoi City People's Committee authorized the plan for a safe vegetable network towards 2020, the area for vegetable production in the whole city is more than 16,276 ha, of which 151 areas with a total area of more than 6,644 ha (average 44 ha/region) concentrated on safe vegetable production is. Prior to this plan, the area allocated for safe vegetable production in the city was 5,044 ha, with an output of nearly 400,000 tons per year, meeting 40% of Hanoi's demand. The area certified by VietGAP standards is 521.6 hectares, and approximately 50 hectares are certified with organic standards. Moreover, the attained economic efficiency of growing safe vegetables is 10 to 20% higher than that of conventional vegetables, the production value is from 300 to 500 million VND/ha/year, and about 1,200 ha is worth 1 billion VND/ha/year due to vegetable production in greenhouses, net houses, and off-season cultivation. The city's leading suppliers for safe vegetable production are cooperatives and enterprises that participate in producing safe vegetables (Hanoi Department of Agriculture and Rural Development N.A.).

Although it takes more investment and training for farmers to grow safe vegetables (MARD 2008), the consumption of safe vegetables has not increased as expected. In 2018, the Hanoi Division of Plant Protection, which belongs to Hanoi Department of Agriculture and Rural Development, stated that safe vegetables have 40% of the total vegetable consumption in Hanoi, however, only 20% of safe vegetable consumers are frequent consumers purchasing safe vegetables. Many farmers who produce safe vegetables face difficulties selling their produce in supermarkets and vendor stores. Only a few vegetable buyers choose safe vegetables due to their high prices. In addition, according to usual observations, there is another issue that some safe vegetables, whose prices are ranged only from 1,000 to 2,000 VND (0.041 - 0.083 USD), which is slightly higher than the conventional counterpart in many supermarkets and online stores in various e-commerce platforms, could not get an as good amount of consumption as traditional vegetables'. Moreover, recently there has been a conspicuous issue: it has been detected that plenty of agricultural products with the VietGAP logo on the package as Vietnamese vegetables come from China and do not undergo any safe vegetable production or verification process. In short, they are non-VietGAP products sold to retail markets and supermarket chains like Bach hoa xanh and Winmart - famous chain stores selling fresh vegetables (Mai et al. 2022), under the VietGAP standard label. Therefore, there might have been a lack of recognition and belief for GAP vegetables among consumers.

When a pandemic breaks out, people tend to follow a certain diet to protect themselves and enhance their immune system for their health (Rodríguez et al. 2020). The demand for food containing biologically active ingredients or food groups with vitamin supplements, such as fruits and some vegetables, increased. A survey conducted in Vietnam found that lockdown during the early part of the severe phase of the Covid-19 pandemic caused 45% of consumers to stockpile food at home, a higher number than before, 50% of whom reduce the frequency of going to places like supermarkets, grocery stores to buy goods and 25% of people limit eating out activities (Nielsen 2020). Aside from the lockdown period, housewives are also gradually reducing the habit of going to the market to buy daily food, and the 9X generation is more interested in green living (Thuy 2020). They tend to spend more time in supermarkets, and are more likely to find VietGAP vegetables intentionally or by chance. Besides, an investigation by MARD indicated that in the early phase of social isolation in the second quarter of 2021, there were 3.7 million tons of fruits and vegetables in 26 provinces that needed support in terms of circulation to be consumed. The situation is similar in many other countries, such as India, where a significant number of vegetable farmers reported a decrease in prices, with more than 80% of farmers reporting reductions by more than half and a drop in farm income for 90% of farms, of which 60% experience a decrease of more than 50% (Harris et al. 2020). As a result, both providers and consumers sought online channels to sell and buy products with the government's support. On July 21st, 2021, the Ministry of Information and Communications approved the plan to facilitate agricultural production on e-commerce platforms, with the view of reinforcing the digital economy, agriculture, and rural areas. Interviews conducted by government electronic news for Hanoi and Ho Chi Minh City inhabitants about GAP vegetables also implied the rising awareness of GAP vegetables among consumers, thanks to the exposition to e-commerce platforms, which seems to be a bright prospect for VietGAP vegetable consumption, considering the low infrequent buyer percentage of safe vegetable in 2018. Therefore, this study aimed to find out the determinants of consumers' intention to buy VietGAP vegetables in the new era when shopping is done in a more accessible way.

Several studies focused on the variables affecting consumers' intention to buy fresh foods, both domestically and internationally. In Slovenia, purchasing intention for fresh fruits and vegetables is affected by the availability of retail stores, consumers' income, consideration of health and environment, and visual appeal (Kuhar and Juvancic 2010). In Malaysia, two factors: health consciousness and perceived value influence positively the intention to buy organic products (Shaharudin et al. 2010). There is a reverse relationship between the perception of quality and risk affecting purchase intention (Alamsyah and Angliawati 2015). Health concerns, perceived quality, health security concerns, beliefs, and higher price for safe food products affect the behavior of consumers in the UK (Dickieson et al. 2009). Perceived organic information positively impacts organic food consumers' attitudes and trust in Indonesia (Secapramana and Katargo 2019). In Tanzania, three components of TPB and health consciousness proved to be the criteria for the extent to which consumers are willing to buy organic foods when knowledge plays as a moderator for the impact of these determinants on buying intention. However, this study showed an insignificant relation of perceived behavioral control with purchasing intention in Kenya (Wang et al. 2019).

Recent research on safe vegetable awareness and consumer behavior in Vietnam, including GAP and organic vegetables, has been conducted. Determinants of the buying intention for safe vegetables in Gia Lam and Long Bien Districts, Hanoi were investigated (Do et al. 2015). It showed that consumers' perception of safe vegetables is still limited, and the intention to buy relies on factors such as age, safe vegetables buying experience, perception of safe vegetables, awareness of what safe vegetables bring about, and sensitivity level about price. Another study claimed that Hanoi consumers prioritize the presence of safety labels or logos on the products, followed by traceability information, availability, and price (Thai et al. 2015). Significant effects of attitudes towards the environment, perceived value, concern about health, knowledge of organic foods, and subjective norms on the attitudes toward organic foods and the purchase intention of organic food consumers in Hanoi and Ho Chi Minh City (Nguyen 2014). Besides, there is a highly positive connection between the attitudes toward organic food and customers' intention to buy organic foods. TPB was used in a study on the following factors influencing safe vegetable buying intention of Ho Chi Minh City consumers: trust in safe vegetables and distributors, concern about health and environment, subjective norm, attitude towards price, and attitude towards convenience (Pham 2016). The attitude towards convenience did not influence the intention to buy. Only attitude towards price has a negative correlation with the intention, and the rest factors have positive impacts with the descending order of impact level: trust, concern, and subjective norm. The four factors that affected safe vegetable purchasing intention, arranged by level decreasing order: concern about health and safe vegetable quality, subjective norm, environmental concern, and price (Ha et al. 2017). The inexistence of the direct impact of a higher level of awareness about organic foods and the information provided on organic food labels and a more positive attitude towards organic food has also been explored (Huynh and Ha 2021).

In summary, most international studies imply that vegetable attributes like price, freshness, appearance, color, taste, nutrition value, and availability statistically influence significantly the intention to buy safe or fresh vegetables. On the other hand, domestic studies tend to prove that consumers' consciousness, perception, and experience have influences on the intention. Besides, most studies have not mentioned much about online consumption, which recently emerged actively in the marketing channel of vegetables.

CONCEPTUAL FRAMEWORK

Intention to behave is influenced by three factors: belief in behavior, belief in standards, and belief in control (Ajzen 2002). When these become intense, people are more likely to perform a particular action. The Theory of Planned Behavior (TPB) is a theory growing from the base of the Theory of Reasoned Action (TRA) which emphasizes the importance of intention in driving behavior (Ajzen 1991; Ajzen and Fishbein 1975). It represents the extent to which people will put effort in or how much attempt they intend to make to perform a particular behavior. The TPB suggests that intention is controlled by: (1) attitude, (2) subjective norm, and (3) perceived behavioral control. Researchers have used widely this theory to explain the purchase intention of various food products (Table 1).

Although there have been some studies about determinants of consumer's VietGAP vegetable buying intention, the number of research in this field is still limited, and they did not consider e-commerce platform characteristics which consist of shipping service, promotion, superior availability as well as VietGAP vegetable attributes that were related to vegetable online consumption channel (storage, shipping service and the like). In the online consumption explosion era, those features must be taken into account to find out elements that can manipulate the intention to acquire VietGAP vegetables of consumers in this study. Therefore, based on past studies applying TPB and the necessity to consider more factors, this study suggested the conceptual framework and its component descriptions as showed in Table 2.

A positive attitude is an important premise that promotes the intention to buy Teng and Wang (2015). The more favorable one's attitude is toward behavior, the more likely that the individual intends to perform the behavior. For subjective norm, it is determined by the belief that influencers think that this individual should perform the behavior (Ajzen 2002). Perceived behavioral control is defined as how a person perceives their action based on their abilities (Sentosa and Mat 2012). Knowledge is a tool to gauge the perceptions regarding the level of understanding that consumers have (Chen 2008).

Traceability in vegetable production, as defined by EC regulation 178/2002 is the ability to follow a vegetable product through all phases of its production and distribution. Even though Vietnamese government introduced food the concept of traceability in safety laws ever since 2010, among consumers, there is a deterioration of preference on traceable foods in general due to the harmful food context of Vietnamese food safety (Dang et. al. 2020). Li and Nguyen (2015) indicated that traceability is a factor that reduces uncertaintie and raise consumer's intention to purchase as even repeaters. This study includes traceability as one factor to be tested whether there exists an influence on intention.

In Vietnamese families, it is conventionally assumed that women are responsible for the family's three meals a day and therefore become the food-purchasing decision-makers. However, those who decide what to eat and what to buy are not necessarily those who actually buy, and the decision will differ depending on who an individual lives with. As a result, the consciousness, and attitudes of consumers vary based on multiple socio-demographic features. Additionally, during the pandemic, shopping behavior of consumers has changed drastically, in terms of location, quantity, and frequency. In this study, the authors agree to choose whether living with family, cooking for the family, buying food for the family, measured as dichotomous data. For frequency of purchase, the authors intended to test this variable using Likert scale, however, the test result showed to have unstable change in influence on intention among the levels of frequency rather than every day. Therefore, the authors tried to group those into one and make this variable dichotomous data. Moreover, the authors take experience of buying VietGAP products as an independent variable.

Table 1. Conceptual framework description regarding consumers' intention to purchase

Variables	Description
Attitude	Individual's attitude is the positive or negative feeling of the individual about buying VietGAP vegetables, whether it is positive or negative: *Belief (an individual's faith on various aspects regarding VietGAP vegetables and VietGAP vegetable production)*, Domestic product and producer consciousness, Environmental concerns, Health concerns
Perceived behavioral control	An individual's perceived ease or difficulty buying VietGAP vegetables
Subjective norm	An individual's perception about buying VietGAP vegetables, which is influenced by the judgment of significant others
Awareness	An individual's recognition ability of VietGAP vegetables under various aspects
Knowledge	An individual's actual understanding extent about VietGAP and VietGAP vegetable about various aspects
Attributes	VietGAP vegetables' features: *Price*, *Quality*, *Appearance and packaging*, *Information and traceability*, *Availability*
Demographics	An individual's private information: *Gender*, *Age*, *Income level*, *The cooking role for family*, *Religion*
Intention	An individual's likelihood to buy VietGAP vegetables, considering its being influenced by all other factors

Source: Adapted from the original model by Ajzen (1985, 1991): Compiled from Ajzen and other past studies' models; Attitudes, perceived behavioral control, subjective norm intention (Ajzen 1985); Knowledge (Secapramana and Katargo 2019); Price, availability (Le 2017); Appearance (Nguyen 2012); Quality, information and traceability (Dickieson and Arkus 2009).

METHODOLOGY

Study area. This study conducted an online survey of Hanoi and Ho Chi Minh inhabitants to capture the fundamental demographic features and their attitudes toward various aspects of VietGAP vegetables**.** Hanoi serves as Vietnam's capital city. It consists of twelve urban districts, one town at the district level, and seventeen rural districts. With an area covering 3,359.82 km^2, after Ho Chi Minh City, Hanoi is the country's second-largest cultural and political hub. It is situated in Northern Vietnam. In the early stage of the safe vegetable program, Hanoi was picked as the first experimental spot for the program due to its main market of vegetables in the country. Based on the information provided by MARD (2015), the demand for green vegetables in the capital city amounts to 950,000 tons per year. Ho Chi Minh City, the country's largest city, possessing a population of around 9.17 million, ranking first above Hanoi, has 8.33 million residents in 2021, occupying 2,061 km^2 in the Southeast region. Both cities are major economic centers of the country, representing the economy of the two areas. They also possess a large population and high average income and attract people from other provinces. With a high economic growth rate, these cities have a significant number of consumers with great demands for vegetables and, as a result, have become places where vegetables coming from many places are distributed. Moreover, consumers in these cities are incredibly up-to-date and highly concerned about smart expenditure and health; most people in the cities are also sensitive to the development of technology. Therefore, conducting the research will be advantageous, and the research results will be meaningful.

Data selection and collection. The survey was conducted during January 2022 and November 2022. The data collection is conducted through an online questionnaire survey, targeting both physical store-visiting and online consumers, residing in the two areas. The questionnaire was distributed randomly to over 300 people over 18 years old. According to World Health Organization, "young people" are defined as individuals whose age is from 10 to 24, and Ha and Ha (2017), in the study of consumer attitude and intention to buy VietGAP vegetables, decided to take 18-25 as one category, moreover, from 26 years old, an individual is considered having changed to another stage of life, therefore, collected age data will be recoded into two groups which are young people ($\leq$ 25 years old) and older people. What is more, the frequency of buying vegetables is inquired, and buying as a daily habit is considered one category besides the rest. Principal component analysis and ordinal logistic regression were used to evaluate the model's reliability and determine the latent variables as well as the available variables in the hypothetical model that influence the buying intention of VietGAP vegetables. Besides, the correlation analysis was also conducted. The measurement applied the 5-point Likert scale: (1) strongly disagree, (2) disagree, (3) neutral, (4) agree, (5) strongly agree. The authors used Ordinal Logistic Regression (OLR) for the the dependent variable Intention (INT: I am very likely to buy VietGAP vegetables) with those 5 points of Likert scale.

Selection of sample size: A sample size of at least 100 is recommended, with the smallest sample having an expected ratio of 5 observations per variable (Hair et al. 2010). The sample size should therefore be greater than or equal to 100 and should be calculated as $n \geq 5k$ (where k is the number of variables). Initially the study aimed for a large set of variables, however, due to the spatial barrier, the survey obtained a smaller number of variables in total than expected in the study. There are 301 variables collected in the questionnaire, which means that the maximum number of independent variables that can be included in the analyses is 60. Representative observed variables were chosen in each construct and in the end, 59 variables were selected for further evaluation.

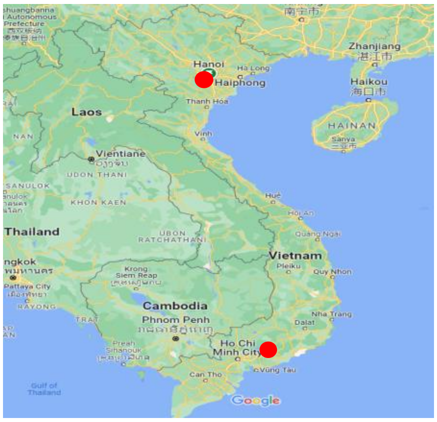

Fig. 1. Map of the study area, Hanoi and Ho Chi Minh City

Method of analyses. Cronbach's Alpha is utilized to evaluate measurement scales' reliability. The greater the Cronbach's Alpha coefficient, the stronger the correlation between the scale's variables. However, many variables on the scale will not differ if Cronbach's Alpha is this high (>0.95), indicating that they measure the same study concept content (Nguyen 2013). Cronbach's Alpha coefficients of 0.8 to 1 indicated a good scale, 0.7 to almost 0.8 indicated a usable scale, and > 0.6 indicated an acceptable scale in situations where the topic is novel or studied in a novel setting (Hair et al. 2010), with corrected item - total correlation smaller than 0.30, the item is unacceptable (Cristobal et al. 2007). Besides, Cronbach's Alpha coefficient if deleting an item must be smaller than Cronbach's Alpha coefficient of the scale (Hoang and Chu 2008).

Principal component analysis: Conditions to consider whether to keep or eliminate variables are Factor loadings, Eigenvalue, and Percentage of variance. A variable is kept when the loading score exceeds 0.3, Eigenvalue surpasses 1, and the Percentage of variance is over 50% (Anderson and Gerbing 1988).

Ordinal logistic regression model: Consumers' intention to purchase VietGAP vegetables is measured as an orderly 5-point categorized dependent variable for the statement: 'I am likely to buy VietGAP vegetables', including strongly disagree, disagree, unsure, agree, and strongly agree. This study employs a multivariate ordered logistic model to examine the key influencing factors. The authors select the logistic model for the reason that its variables may not satisfy the demands of normal distribution. The dependent variable, demonstrated by Y, is continuous with an ordinal scale, categorized as j = 1, 2, 3, …, J, then the cumulative probability of Y less than or equal to a certain category j = 1,…, J-1, is represented by $P(Y \leq j)$, with $P(Y \leq J) = 1$ (Nguyen, 2019). The odds ratio of Y being less than or equal to a specific category can be interpreted as $\frac{P(Y \leq j)}{1-P(Y \leq j)}$ for j = 1, …, J-1 since $P(Y > J) = 0$, or $1 - P(Y \leq J) = 0$ (Parry, 2020).

Log odds is known as the logit, and it is common to interpret coefficients via odds ratio, in SPSS, the odds ratio is figured out by taking exp(β).

$$\text{Logit}[P(Y \leq j)] = \ln\left[\frac{P(y \leq j)}{1-P(y \leq j)}\right] = \alpha_j + \beta X \qquad j = 1, \ldots, J\text{-}1 \qquad \text{(Nguyen 2019)}$$

This logistic model here includes the intercept parameters α_j (also termed the cutting point, $\alpha_1 \leq \alpha_2 \leq \ldots \leq \alpha_{J-1}$), and the coefficient β for the independent variable vector X, where covariates X_i are the p-dimensional vectors of explanatory variables (i = 1,2…., p), in this case, X_i denotes any index that has an impact on consumers' purchasing intention of VietGAP vegetable. This study considers Y as consumers' VietGAP vegetable buying intention with five categories above, and 19 independent variables as part of the analysis. Table 8 shows how the categorical variables are recoded before being incorporated in the regression analysis.

Table 2. Item coding

Item	Categories	Code	Item	Categories	Code
Vegetable purchase frequency (VPF)	Less frequent	0	Likert scales	Strongly disagree	1
	Every day	1		Disagree	2
Age	Older	0		Neutral	3
	Young	1		Agree	4
Live with family; Cook for family; Food purchase for the family; GAP buying experience				Strongly agree	5
	No	0	Gender	Female	0
	Yes	1		Male	1

Source: Survey, 2022

RESULTS AND DISCUSSION

Characteristics of the respondents. The number of attained survey respondents is originally 334. The screening and filtering helped the authors to eliminate 33 invalid cases, the remaining 301 (90.1% valid cases) are used for analysis, as shown in Table 3. The majority of respondents are female, with almost 59% of the respondents. Respondents' age ranges from 18 to 36 years old, with an average of 24.07 years old; most are single because the questionnaire was distributed online through social network groups, where active users are primarily young, already working, or still studying in college. Regarding religion, respondents are divided into two main groups: Buddhists, accounting for approximately 48%, and the rest, including Catholics. Also, data implied that more than three-quarters of respondents have experience buying VietGAP vegetables. Income was measured in individual and monthly amounts because of the online survey and the fact that the majority are undergraduates, therefore, the revenue of respondents is hugely different from one another's. Some respondents are students living on family support, and their income was noted as zero. Vegetable purchase frequency is measured on two scales (Table 3), with around 17% of respondents shopping daily for vegetables.

Table 3. General profile of respondents (n=301)

Item	Code	No	%	Item	Code	No.	%
Gender	Female	177	58.8	Cooking responsibility	No	111	36.9
	Male	124	41.2		Yes	190	63.1
Religion	No religion	132	43.9				
	Catholicism	24	8.0	Food purchase responsibility	No	147	48.8
	Buddhism	145	48.2		Yes	154	51.2
Marital status	Single	255	84.7				
	Married	46	15.3	Vegetable purchase frequency (VPF)	Less frequent	251	83.4
Education level	High school	57	18.9		Every day	50	16.6
	Undergraduate	188	62.5				
	Graduate	56	18.6	Age (as categories)	Young	208	69.1
Employment	Unemployed	85	28.2		Older	93	30.9
	Work part-time	91	30.2	VietGAP vegetable purchase experience	Inexperienced	72	23.9
	Work full-time	125	41,5		Experienced	229	76.1

Living status	Outside home	110	36.5
	With family	191	63.5
	Value range		Mean
Age	18-36		24.07
Income (VND)	0 – 30,000,000		7,897,009.97

Source: Survey, 2022

Reliability of the model. Throughout the survey, 59 measured variables are grouped in 13 different scales. Cronbach's Alpha of the scale 'Price' (measuring consumer's perception of VietGAP vegetables' price) is 0.854, which is acceptable (> 0.6), corrected item - total correlation index of all the included variables are all greater or equal 0.587 (> 0.3), and the case of Cronbach's Alpha coefficient increasing when one variable is excluded does not occur. Therefore, the scale 'Price' is qualified with four variables.

Cronbach's Alpha of the scale 'Quality' - measuring consumer's perception of VietGAP vegetables' features regarding quality - is 0.810. This scale's corrected item - total correlation of all the composing variables has the smallest value at 0.559. Also, Cronbach's Alpha of the scale will decline if one item is eliminated. Thus, four observed variables are preserved for the subsequent analyses. In the same way, scales 'Appearance and packaging' - assessing an individual's perception of VietGAP vegetables' exterior features, 'Information and traceability' - measuring a person's perception of information sufficiency, 'Availability' which measures an individual's perception of accessibility, 'Belief' that measures an individual's beliefs on products and producers, 'Domestic products and producers' consciousness' - measuring consumer's domestic product support, 'Environment concern' - estimating one's perception of VietGAP vegetables' effect on the environment, 'Health concerns' - measuring the perception of VietGAP vegetables' effect on consumer and producer's health, 'Perceived behavioral control', 'Awareness', 'Knowledge', and 'Subjective norm' are respectively qualified with the number of variables as described on the tables.

To sum up, the reliability test result in Table 4 showed that all the scales meet the requirements in terms of reliability with Cronbach's Alpha equal to or higher than 0.6, and all the corrected item - total correlation values equal or exceed 0.3, hence, it is acceptable to proceed to principal component analysis.

Table 4. Reliability test result

Item	Number of variables	Cronbach's alpha	Corrected Item-Total Correlation minimum
Price	5	0.854	0.587
Quality	4	0.810	0.559
Appearance and packaging	6	0.881	0.609
Information and traceability	5	0.875	0.634
Availability	4	0.849	0.668
Beliefs	4	0.902	0.724
Domestic products and producers' consciousness	5	0.887	0.631
Environment concern	4	0.867	0.706
Health concerns	4	0.846	0.516
Perceived behavioral control	6	0.867	0.453
Awareness	4	0.848	0.648
Knowledge	4	0.859	0.662
Subjective norm	4	0.820	0.564

Source: Data analysis by SPSS using survey data

Principal component analysis. Principal component analysis with the varimax rotation method for 59 variables (Kaiser 1958) is applied to extract multiple variances from the measured variables with the smallest number of components to serve the forecasting objective (Dunteman 1989; Hair et al. 2010). The result showed that four variables were eliminated from the model due to unqualified loading scores. After processing the analysis results, the rotated component matrix table showed the adjusted model (Table 5). There are 55 observed variables divided into 12 components, with relatively high total cumulative variances explained (70.191% > 50%), component scores are all above 0.5 (KMO = 0.934) (Kaiser 1974), the p-value of the Bartlett test is 0.000 (<0.05). Therefore, the modified model with 12 components and 55 variables is appropriate for further analysis.

Based on the theoretical basis and existing observed variables in each group, the scales of independent variables are adjusted as follows:

The component on Appearance and packaging remained six variables from the original scales' Appearance and packaging', describing the consumer's view of the product's exterior. The Promotion of domestic products component, with five variables, represents consumers' stimulation of advocating domestic product which is highly recommended by the country. Component Traceability has five items from the scale 'Information and traceability', which is how an individual feels about the portraited information and QR code accessibility. Then the Price evaluation component adopts five items from the scale 'Price', which are all about the matter of price when it comes to VietGAP vegetables, in consumers' viewpoints. Following is the Awareness component, containing four items, which reflect the ability of ordinary consumers to realize VietGAP vegetables in daily life. Component Perceived behavioral control owns five variables about the scale' Perceived behavioral control', as its definition, this component wraps up the personal feeling of difficulty enacting the purchasing behavior. Component Beliefs on VietGAP production, comprising four items taken from the 'Belief' scale, lists consumer belief in various aspects of VietGAP vegetables. Component Environmental concern, having four observed variables from the same scale, is the buyer's sense of environment when consuming VietGAP products. The Attained Knowledge of VietGAP component is the assemble of four items of the 'Knowledge' scale, showing the understanding of consumers on VietGAP vegetables, based on what they have learned or what they have themselves experienced. Next, the tenth component, Availability of VietGAP vegetables, consists of four variables in the scale 'Availability'. It reflects how people get access to the presence of vegetables. After that is the Subjective norm component, which involves the belief of the consumer about whether the purchasing behavior are affected by the significant others' approval or disapproval. The last component, Concern about health, has three items on the 'Health concerns' scale and two on the 'Quality' scale, and all mention how one pays attention to the effect of VietGAP on health care. Consequently, the author combined them as one group mentioning Health concerns.

Table 5. Rotated component matrix of principal component analysis.

Component 1: Appearance and package (X_1)	*Subtotal variance: 34.244*	**Score**
VietGAP vegetables bring safety image		0.747
VietGAP vegetables packaging is simple		0.743
VietGAP vegetables stand out when being placed near conventional vegetables		0.708
VietGAP vegetables look fresher than conventional vegetables		0.691
VietGAP vegetables look exactly like what was promoted on TV		0.644
Appearance is important for me when I buy vegetables		0.545
Component 2: Promotion of domestic products (X_2)	*Subtotal variance: 6.625*	**Score**
By consuming domestic vegetables, I can indirectly help farmers to improve their income		0.787
People are encouraged to consume domestic products by the government		0.765
Consumption and feedback are the key to production improvement		0.722

Domestic products bring creditable feelings	0.653
Buying VietGAP vegetables is a way of advocating the domestic products	0.652
Component 3: Traceability (X_3) *Subtotal variance: 4.930*	
QR codes on VietGAP vegetable products are readily found on the package	0.812
By scanning QR codes on VietGAP vegetable products, customer can access to necessary information	0.742
VietGAP vegetable logo, package provide adequate information regarding products	0.731
Association that managing GAP participants often update statistics about numbers, places, products of participants	0.730
VietGAP vegetables' logo and package provide accurate information about product	0.610
Component 4: Price evaluation (X_4) *Subtotal variance: 4.283*	
VietGAP vegetables being of higher price is also an indication of its being tastier	0.796
VietGAP vegetables are expensive	0.755
I think paying highly for VietGAP vegetables is necessary	0.726
I think price does not matter when I buy VietGAP vegetables	0.653
I usually applied vouchers when I buy VietGAP vegetables online	0.567
Component 5: Awareness of VietGAP (X_5) *Subtotal variance: 3.359*	
On average, people in Hanoi once heard about VietGAP vegetables and saw it	0.797
Convenience stores/Supermarkets have special corner for promoting VietGAP vegetables	0.769
Due to the effect of Covid-19, the number of people seeking VietGAP vegetables is increasing	0.745
Agriculture promoting TV programs positively widen the image and information about GAP vegetable to people	0.745
Component 6: Perceived behavioral control (X_6) *Subtotal variance: 2.979*	
I feel confident to buy VietGAP vegetables because the government is supporting VietGAP products	0.683
I find it easy to consume VietGAP vegetables because I can access full information through its QR code	0.628
I find it easy to buy VietGAP vegetables because I can effortlessly distinguish it from conventional vegetables	0.618
I find it comfortable to buy VietGAP vegetables because e-commerce basis provides me a lot of promotion, deals	0.581
I feel confident to buy VietGAP vegetables because the government stimulates people to consume VietGAP products	0.559
Component 7: Beliefs on VietGAP production (X_7) *Subtotal variance: 2.778*	
I believe that large corporation has a role on leading farmers to produce VietGAP vegetables in terms of technique	0.782
I believe in VietGAP vegetable advertisements	0.739
I believe VietGAP vegetable sellers are guaranteed with quality certificate	0.720
I believe that large corporation follow the GAP instruction to produce vegetables	0.714
Component 8: Environmental concern (X_8) *Subtotal variance: 2.745*	
Consuming VietGAP vegetables is a means of protecting animal habitat	0.763
It is important to produce environmentally friendly vegetables	0.753
Green consumption is highly recommended by the government to protect the environment	0.717
Advertisements regarding environmental protection usually include VietGAP	0.716

Component 9: Attained Knowledge of VietGAP (X_9)	*Subtotal variance: 2.302*	**Score**
To understand the standard is essential before consuming		0.768
Due to Covid-19, many consumers have to shift to online shopping, thus learn more about GAP because GAP vegetables are highly promoted there		0.720
The government is widening the GAP knowledge for farmers		0.674
I have learnt about VietGAP standard		0.663
Component 10: Availability of VietGAP vegetables (X_{10})	*Subtotal variance: 2.080*	
The e-commerce platform I use provides VietGAP vegetables		0.684
VietGAP vegetables are available in places that are not far from my house		0.680
The place where I usually buy VietGAP vegetables provide all kinds of vegetables that I need		0.662
The shipping service for VietGAP vegetables from online shops is fast and 24/7		0.650
Component 11: Subjective norm (X_{11})	*Subtotal variance: 2.008*	
People who are close to me agree that spending much on VietGAP vegetables is wasteful		0.767
My friends suggest that I consume VietGAP vegetables		0.696
A lot of people I know are consuming VietGAP vegetables regularly		0.659
Promotions on supermarket stimulate me to buy VietGAP vegetables		0.614
Component 12: Concern about health (X_{12})	*Subtotal variance: 1.857*	
VietGAP vegetables are good for consumer's health		0.655
Planting VietGAP vegetables reduces the burden on farmers' health		0.637
Consuming VietGAP vegetables raise the awareness standard of health care		0.584
Chemical residuals in VietGAP vegetables are within permitted quota		0.580
Farming practice for VietGAP vegetables is well abided by the farmers		0.533
	Total variance: 70.191	

Source: Data analysis by SPSS using survey data

The revised model, which contains 12 components, is then put into a re-reliability test to confirm the constructs' validity. All the new scales meet the requirements in terms of reliability with Cronbach's Alpha above 0.6, the whole corrected item - total correlation indexes go beyond 0.3, with no increasing Cronbach's Alpha in case of deleting any item. Therefore, it is suitable for logistic regression analysis (Table 6).

Table 6. Reliability test for revised model's scales

Component	Name	Number of variables	Cronbach's alpha	Corrected Item-Total Correlation minimum
Appearance and package	X_1	6	0.881	0.609
Promotion of domestic products	X_2	5	0.887	0.631
Traceability	X_3	5	0.875	0.634
Price evaluation	X_4	5	0.854	0.587
Awareness of VietGAP	X_5	4	0.848	0.648
Perceived behavioral control	X_6	5	0.883	0.677
Beliefs on VietGAP production	X_7	4	0.902	0.724
Environmental concern	X_8	4	0.867	0.706
Attained Knowledge of VietGAP	X_9	4	0.859	0.662
Availability of VietGAP vegetables	X_{10}	4	0.849	0.668
Subjective norm	X_{11}	4	0.820	0.564
Concern about health	X_{12}	5	0.844	0.584

Source: Data analysis by SPSS using survey data

Ordinal Logistic Regression. In this logistic model, there are four cut-points (because the dependent variable is divided into five intervals according to ordinal scales), which are all statistically significant (Table 9, dependent variable). Therefore, it is necessary to measure Intention with five scales as it is. Independent variables are seven components that are attained from Principal Component Analysis X_i; Income; and Vegetable Purchase Frequency. Since Age and Income are highly correlated (Table 7), regression analysis was done separately for Income and Age as independent variables, otherwise, there will be a collinearity phenomenon.

Table 7. Pearson correlation analysis

		Income	Age
Income	Sig. (2-tailed)		0.000
	N	301	301
	Pearson correlation	1	-0.671**
Age	Sig. (2-tailed)	0.000	
	N	301	301
	Pearson correlation	-0.671**	1

Source: Data analysis by SPSS using survey data

The result of regression analysis showed that components X_i (i = 1; …;12), except for X_5 Awareness of VietGAP, Vegetable purchase frequency at everyday level, with Sig. (p-values) < 0.05, are statistically significant, with positive signs (the sign of coefficients), implying the rising intention if one has a habit of going shopping regularly at least once a day. While in the status of living with family, the coefficient is negative, meaning the reduction in intention. There is a significant positive influence with *Income*, whereas the list including *Age* figured out a significant negative influence. The estimated value, the Coef. in Table 9, of the Ordered Logit model's sign and significant level indicated the influence magnitude of independent variables on the dependent variables, the Odds ratio indicates how much one unit change in an independent variable will change the dependent variable Intention. The author picked those items with odds ratio greater than 2.00 to put into discussion, however, the way of explanation can apply to all the significant factors X_i.

In the case of *Age* as an independent variable, the model I in Table 9 suggests that a one-unit increase in *Appearance and Packaging* leads to 0.715 times in the ordered log-odds – the coef. value, thus 2.04 times – the odds value, more intention to buy, given that the other variables are constant, implying the positive change in VietGAP vegetable package, exterior, or consumers' positive outlook on those features will attract them to buy. A unit increase in Promotion of domestic products is expected to have 0.988 ordered log odds, thus 2.69 times more intention to buy, on the condition that all of the other variables in the model are stable, this explains consumers are more willing to buy if they percept VietGAP vegetables as domestic products.

The same holds for all the other nine components in the model. When traceability and Price evaluation of VietGAP vegetables increases by one unit, there are 1.75 and 2.09 times and the intention to buy VietGAP vegetables. Apparently, as in other studies on consumer behavior, price is frequently a determinant of intention to buy a product, and this study indicated the positive view on price over the overall product – VietGAP vegetable, will raise the desire to buy. Similarly shown by the model II in Table 9, one unit rise in Perceived behavioral control, Beliefs in production, Availability of products, and Health concerns, will cause 2.89, 2.28, 2.38, and 2.50 times more intention, respectively. Consumers are more likely to purchase VietGAP vegetables if they find it easy, and conveniently available to do so, if they have a decent understanding of production processes, or if they treat the products as an agent that produces positive effects on healthcare. When consumers shop for vegetables daily, they are expected to have 6.26 times more intention to buy VietGAP products. However, if an individual is supposed to live with family or is under 25, one's intention is expected to be almost halved, to 0.56 or 0.48 times, respectively, meaning that people tend to have less intention to buy VietGAP products if they live with family members, or as they grow.

When analyzing *Income* as an independent variable, Table 9 Model II shows that a unit increase in *Promotion of domestic products*, the ordered log-odds is inclined to be 0.934, thus bringing on 2.54 times more intention to buy if all other variables remain unchanged. Likewise, one unit rise in *Perceived behavioral control*, *Beliefs in production*, *Availability of products*, and *Health concerns* will respectively lead to 2.83, 2.33, 2.34, and 2.50 times more intention. If an individual is supposed to live with family, that person's intention is expected to be almost halved to 0.53, provided that the other variables are changeless.

Consumers who shop for vegetables daily are expected to have 6.81 times more intention to buy VietGAP products. With a one million VND (approximately 41 USD) increase in *Income*, there will be about 1.12 times in the intention, slightly higher. This is consistent with other studies on consumer behavior, the more consumers can earn, the more likelihood that they will buy the products.

In general, there are eleven components and the variable of buying frequency at the everyday level, whose influences on the intention are positive. In terms of components' influence magnitude, it can be inferred that going shopping every day has the highest impact on one's intention, followed by *Perceived behavioral control*, *Promotion of domestic products*, *Health concerns*, *Availability*, and *Beliefs in production*.

On the other hand, with a negative sign on the estimate of living with family, it can be predicted that people living with family tend to have less intention to buy VietGAP vegetables. This might be due to the fact that in many households, the mother is not the only person who is responsible for buying, even though they are the core menu planner. Under many circumstances, other members like children will be requested to go shopping instead. Therefore, the respondents might be in these cases, and they might not be interested in what type of vegetables but what vegetables they should buy instead. Another possible reason behind this is due to the limited source of collected data. Since the survey was conducted through the internet, the respondents somehow share information and influence each other's living styles, therefore, they are somewhat not too different in terms of buying habits. Consistent with many past studies, the more income, the more intention consumers will possess (Ha et al. 2021).

Table 9. OLR result: Estimates processing (with Age/Income)

	Model I (Excluding Income)					Model II (Excluding Age)				
Goodness-of-Fit Test	Chi-Square		Df		Sig.	Chi-Square		df		Sig.
Pearson	1257.556		1173		.043	1201.755		1181		.331
Deviance	397.649		1173		1.000	390.599		1181		1.000
Pseudo R-Square	Cox and Snell		Nagelkerke		McFadden	Cox and Snell		Nagelkerke		McFadden
	.560		.634		.382	.574		.650		.397
	Coef.	Std. rror	Wald	Sig.	Odds	Coef.	Std. Error	Wald	Sig.	Odds
Dependent variable										
[INT = 1]	-8.221	1.097	56.128	.000		-7.118	1.059	45.162	.000	
[INT = 2]	-6.444	.824	61.229	.000		-5.371	.771	48.559	.000	
[INT = 3]	-2.897	.657	19.426	.000		-1.848	.590	9.820	.002	
[INT = 4]	1.901	.634	8.984	.003		3.097	.618	25.081	.000	
Independent variable										
X_1 Appearance and package	.715	.140	25.890	.000	2.04	.627	.143	19.343	.000	1.87
X_2 Promotion of domestic products	.988	.147	45.021	.000	2.69	.934	.149	39.283	.000	2.54
X_3 Traceability	.559	.139	16.264	.000	1.75	.477	.141	11.378	.001	1.61
X_4 Price evaluation	.737	.153	23.206	.000	2.09	.649	.153	17.903	.000	1.91
X_5 Awareness of VietGAP	.264	.139	3.609	.057	1.30	.257	.141	3.326	.068	1.29
X_6 Perceived behavioral control	1.060	.150	50.194	.000	2.89	1.042	.149	48.847	.000	2.83
X_7 Beliefs in production	.824	.151	29.588	.000	2.28	.848	.152	30.969	.000	2.33
X_8 Environmental concern	.440	.140	9.953	.002	1.55	.422	.142	8.855	.003	1.53
X_9 Attained Knowledge	.501	.137	13.370	.000	1.65	.471	.138	11.715	.001	1.60
X_{10} Availability	.865	.150	33.344	.000	2.38	.850	.151	31.749	.000	2.34
X_{11} Subjective norm	.498	.138	12.960	.000	1.65	.481	.140	11.759	.001	1.62
X_{12} Concern about health	.916	.149	37.862	.000	2.50	.917	.149	37.625	.000	2.50
VPF Every day	1.834	.410	19.970	.000	6.26	1.919	.418	21.057	.000	6.81
Live with family	-.580	.287	4.097	.043	0.56	-.637	.293	4.748	.029	0.53
Cook for family	.397	.307	1.671	.196	1.49	.329	.311	1.120	.290	1.39
Food purchase for family	-.225	.313	.516	.472	0.80	-.325	.319	1.039	.308	0.72
GAP purchase experience	-.179	.319	.313	.576	0.84	-.214	.327	.427	.513	0.81
Gender	.112	.275	.167	.683	1.12	.052	.280	.035	.853	1.05
Age	-.729	.345	4.453	.035	0.48					
Income (million VND)						0.109	0.29	13.619	.000	1.12

Source: Data analysis by SPSS using survey data

CONCLUSION

Consumers' intentions are determined by vegetable purchase frequency with positive influence, 11 latent factors, and income. The most influential factors in decreasing order are: vegetable purchasing frequency at every day, perceived behavioral control, promotion of domestic products, health concerns, availability and beliefs in production. However, the status of living together and being at a young age made reversed impacts. In addition, this study found awareness of VietGAP and other demographic features insignificant, and the rest of the latent factors do have effects on the change of intention, although these are not too impressive.

Based on what the paper finds out – the influencing factors, the influence tendency, and the intensity of the influence, the authors suggest a few solutions to improve the intention as follow: To increase Perceived behavioral control, organizing processes in the physical stores, like separating VietGAP vegetables from the others by designing a secluded corner, writing more details on the description boards, and improving the online webpage interface should be done. The printing of packages for better, more prominent, and readily recognizable certification labels needs improving as well. It is vital for the management officers of MARD to improve the image of farming techniques in VietGAP vegetables under the view of their eyes. How VietGAP vegetables are produced, the process, and the supervision, are advisable, too, so that consumers better understand the production and become confident in their behavior.

The government's objective to stimulate Vietnamese to consume Vietnamese products does have a positive influence, hence, giving more promotion for domestic products like local propaganda and photo contests are highly recommended.

Considering health and beliefs on the production positively relates to buying behavioral intention. In order to boost these, local agricultural offices need to further the VietGAP certificate image promotion to people. It can be done better through food programs, nutrition-related programs on television, and online shopping platforms' advertisements. By doing so, the recognition of VietGAP in consumers' eyes will rise. Moreover, the supervision of VietGAP vegetables should be enhanced by sending officers to vegetable-providing farms in order for fake labeling not to happen. Information stated on the label and information that can be traced via QR codes should be more explicit, complete, and trustworthy (for example, growing method, processed procedures, origin, and expiration date), this is very important to increase consumer confidence in the products they may buy.

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