Agriculture and Life Science Division
College of Natural and Applied Sciences

Technical Report

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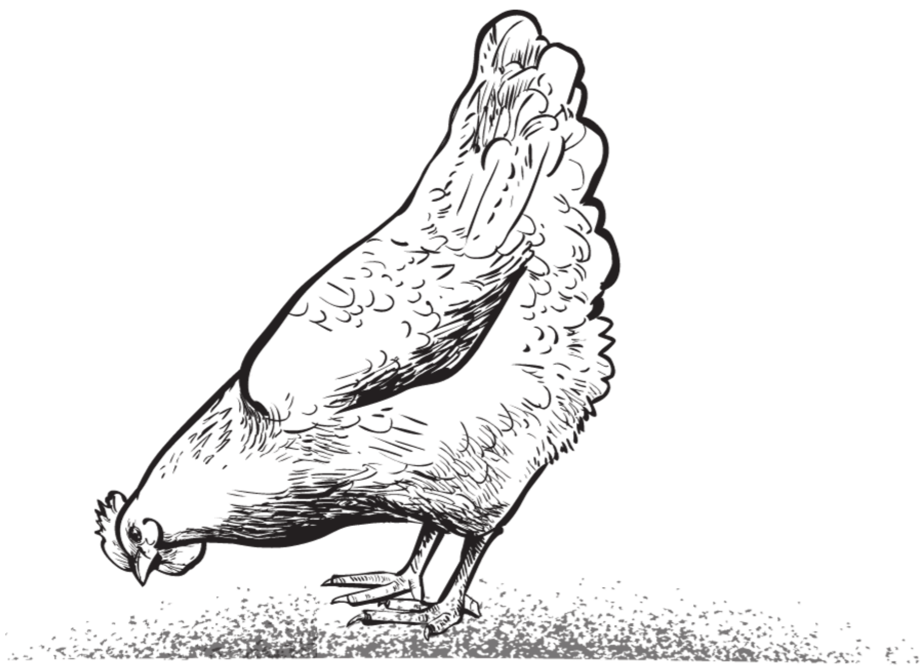
Making Local Layer Feed Using Food Waste in Guam



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Introduction

In Guam, eggs are important protein sources along with chicken, pork, beef, and fish for human diets (Pobocik, et al., 2008). However, most eggs are imported without any large commercial egg farms on the island (Duguies, et al., 2016). The main reason of this scarceness of local egg production is possibly that nearly 100% chicken feeds are imported, and feed cost has increased every year.

Although the island has made some improvement to its waste receivership facilities, Guam still faces a great deal of issues with its generation of waste. Guam produces around 20,000 tons of food waste yearly which makes up about 15% of the entire island's generated waste (Losinio, 2013). If the island were able to divert this food waste away from the landfill and into a more productive alternative such as making farm animal feedstuff and composting, the lifespan of the landfill would surely be lengthened and, in turn, become a more sustainable set-up for Guam.

The research was conducted to examine locally available resources to supplement commercial layer feeds and to determine their effects on egg production and egg quality. Local resources included kitchen scraps, fish scraps, and spent grain (waste from brewing beer) to divert food waste away from the landfill. Waste recovery and diversion is an essential component in moving Guam into a sustainable future. Other local bioresources were multipurpose trees, such as those windbreak trees, and unmarketable excess farm crops. The project aimed to: (1) analyze nutrient contents of potential local bioresources to develop layer feeds and (2) conduct feed trials determining effects of local layer feeds on egg production and quality of eggs. This project was performed with collaboration between the University of Guam and the University of the Ryukyus, Faculty of Agriculture and Graduate School of Agriculture.

STUDY 1:
Nutrient analyses of local resources as ingredients of layer feeds

Materials and Methods

Various resources as potential ingredients of layer feeds in Guam were collected during February 26 to March 19, 2016 (Figs. 1-7) and their nutrient levels and metabolizable energy were determined. Kitchen waste was collected from two local restaurants. Spent grain was obtained from a local beer brewer and coffee ground was collected from two different coffee shops. Fish scrap including some bones was obtained from the Guam Fisherman's Co-Op store. Bio-resources collected from a farm included cassava (*Manihot esculenta*) tubers, local pumpkin (*Cucurbita* sp.), breadfruit (*Artocarpus altilis*), calamansi (x *Citrofortunella* sp.), papaya (*Carica papaya*), eggfruit (*Pouteria campechiana*) fruits and leaves, young tangantangan (*Leucaena leucocephala*) fruits and leaves with green stems, and young moringa (*Moringa oleifera*) fruits and leaves with green stems. In addition, nutrients were analyzed from commercial cracked corn and lime as ingredients of chicken feeds.

All samples were dried at 70°C for 48 hours and ground with a blender prior to determination of metabolizable energy (ME) and nutrients. The ME was calculated according to the methods described by Palić (2012) and Boisen (1997) from the value of the enzyme digestibility of organic matter (EDOM). EDOM was determined by placing 0.5g sample in a 100mL Erlenmeyer flask and adding 25mL of 0.1M phosphorous buffer (pH=6). While mixing sample solutions with a stirrer, 10mL 0.2M HCl was added and adjusted to pH=2 by adding either 1M HCl or 1M NaOH. Then, one mL of solution with 25mg pepsin (enzyme) and 0.5mL of 0.5g chloramphenicol (antibiotic)/100mL ethanol were added to sample. After placing a stopper on the flask, the sample was incubated in a water bath at 39°C for 75 minutes. Ten mL of 0.2 M phosphate buffer (pH=6.8) and 5mL of 0.6M NaOH was added to the sample solution, and pH was adjusted to 6.8 by adding 1M HCl or 1M NaOH. One mL of the solution with 100mg pancreatin was added to the sample solution and incubated at 39°C for 18 hours. The sample was filtered using a glass filter, washed with 10mL 96% ethanol and then with 10mL 99.5% acetone. The air-dried sample was weighed, placed into a muffle furnace at 500°C for 4 hours, and the remaining ashes was then weighed.

The dry matter content (DM), crude protein (CP), crude lipids (EE), crude ash (CA), crude fiber (CF), and nitrogen-free extract (NFE) were determined according to the method by Sudo (2001).

Mineral contents were determined using the inductively coupled plasma atomic emission spectroscopy (Shimadzu Inc.) according to methods described by Isaac (1998). In preparation of sample solution, 0.5g of dry sample in a 50mL beaker was placed in a muffle furnace at 550°C for 4 hours and then in a desiccator chamber for 1 hour. After adding 10mL of nitric acid (1+1) and dissolving the sample for 10 minutes, sample solution was transferred to 100mL volumetric flask, and distilled water was added to make 100mL sample solution. The sample solution was used to determine the concentration of Ca, P, K, Cu, Fe, Mg, Mn, S, and Zn.

Fig. 1. Food waste collected from a local restaurant.

Fig. 2. Spent grain collected from a local beer brewer.

Fig. 3. Sliced unmarketable cassava (*Manihot esculenta*) from a farm.

Fig. 4. Dried bread fruit (*Artocarpus altilis*).

Fig. 5. Tangantangan (*Leucaena leucocephala*) grown at a farm as a multipurpose tree.

Fig. 6. Moringa tree (*Moringa*) *oleifera*).

Fig. 7. Fruits of Moringa (*Moringa oleifera*).

Results and Discussion

The results of nutrient analyses and the metabolizable energy of local materials as potential ingredients of layer feeds are shown in **Table 1.** The crude protein is one of the most important nutrients needed for layer feed. This study indicated that fish scrap contained the most crude protein, followed by kitchen wastes from the two restaurants. A variation was found in the nutrient contents of kitchen wastes between the two restaurants, which appeared to be due to the different types of food served at the two restaurants. From a farm, we were able to obtain some proteins from leaves and young green seed pods of tangantangan and moringa.

Table 1. The percent (%) dry matter (DM), and crude protein (CP), ether extract, crude fat (EE), crude ash (CA), crude fiber (CF), nitrogen-free extract (NFE), and metabolizable energy (ME) on dry matter basis of potential ingredients of chicken feeds. Materials were collected during February 26 and March 19, 2016.

Resources	DM (%)	CP (%)	EE (%)	CA (%)	CF (%)	NFE (%)	ME (Mcal/kg)
Food Waste collected*:*							
Kitchen waste (Restaurant M)	16.8	**21.3**	29.5	5.0	3.2	41.0	4.78
Kitchen waste (Restaurant C)	32.0	**33.0**	16.8	6.4	3.2	40.6	3.90
Fish bones and scrap	44.1	**48.3**	12.3	22.4	0.0	17.0	2.84
Brewer's spent grain	31.7	17.7	4.8	2.9	8.6	66.0	0.41
Coffee ground (Coffee shop J)	37.3	14.0	15.5	1.6	23.4	45.4	2.29
Coffee ground (Coffee shop I)	37.3	14.5	18.9	1.8	25.2	39.6	2.24
From Farm:							
Pumpkin, *Cucurbita* sp.(fruit)	11.9	10.6	1.4	10.0	10.5	67.5	2.64
Breadfruit, *Artocarpus altilis* (fruit)	33.1	4.8	1.4	2.7	3.0	88.0	2.36
Calamansi, x*Citrofortunella microparpa* (fruits)	16.4	8.8	7.8	5.1	9.9	68.5	3.10
Papaya, *Carica papaya* (fruits)	15.6	10.4	7.1	5.2	9.8	67.5	3.29
Eggfruit, *Pouteria campechiana* (leaves)	43.4	11.5	7.0	9.5	17.4	54.6	1.64
Eggfruit, *Pouteria campechiana* (fruits)	45.6	4.0	2.7	1.4	4.6	87.3	3.11
Cassava, *Manihot esculenta* (tubers)	32.3	3.7	2.0	2.6	2.3	89.4	3.70
Tangantangan, *Leucaena leucocephala* (leaves)	43.0	**22.1**	6.6	9.9	16.8	44.5	1.89
Tangantangan, *L, leucocephala* (young seed pods)	81.4	18.7	3.1	4.2	28.8	45.1	1.45
Moringa, *Moringa oleifera* (leaves)	22.4	**19.1**	6.8	9.6	15.0	49.5	2.27
Moringa, *M. oleifera* (young seed pods)	23.9	14.5	3.5	6.0	25.6	50.5	2.00
Commercially Available:							
Cracked Corns (*Zea mays*)	89.4	9.1	5.3	1.5	2.2	81.9	3.84

The results of mineral analysis are shown in **Table 2.** Kitchen wastes from two restaurants contained more K, S, and Ca than Zn, Mg, Fe, and Cu. The contents of P and Mn were very low or not detected.

Higher contents of K were also found in fruits of pumpkin, breadfruit, and papaya, and leaves and young seed pods of moringa. Ca contents were high in the leaves of eggfruit, tangantangan, and moringa.

Table 2. Mineral contents on dry matter basis of locally available resources as potential ingredients of chicken feeds. Materials were collected between February 26 and March 19, 2016.

Resources	Ca (%)	P (%)	Cu (%)	Fe (%)	K (%)	Mg (%)	Mn (%)	S (%)	Zn (%)
***Food Waste collected**:*									
Kitchen waste (Restaurant C)	0.23	0.00	0.00	0.00	0.01	0.12	0.00	0.52	0.11
Kitchen waste (Restaurant M)	0.50	0.00	0.00	0.00	0.01	0.07	0.00	0.40	0.08
Fish bones and scrap	nd[z]	nd	nd	nd	nd	nd	nd	nd	nd
Brewer's spent grain	0.12	0.02	0.00	0.01	0.00	0.18	0.00	0.45	0.01
Coffee ground (Coffee shop J)	0.13	0.03	0.00	0.01	0.00	0.16	0.01	0.13	0.05
Coffee ground (Coffee shop I)	0.09	0.03	0.00	0.00	0.00	0.13	0.00	0.10	0.06
From Farm:									
Pumpkin, *Cucurbita* sp.(fruit)	0.26	0.00	0.00	0.00	0.03	0.11	0.00	0.60	0.14
Breadfruit, *Artocarpus altilis* (fruit)	0.10	0.05	0.00	0.00	0.01	0.06	0.00	0.15	0.03
Calamansi, x*Citrofortunella microparpa* (fruits)	1.07	0.00	0.00	0.00	0.01	0.14	0.00	0.19	0.11
Papaya, *Carica papaya* (fruits)	0.34	0.00	0.00	0.00	0.02	0.14	0.00	0.30	0.12
Eggfruit, *Pouteria campechiana* (leaves)	2.99	0.00	0.00	0.01	0.00	0.28	0.01	0.10	0.18
Eggfruit, *Pouteria campechiana* (fruits)	0.11	0.09	0.00	0.00	0.00	0.04	0.00	0.10	0.03
Cassava, *Manihot esculenta* (tubers)	0.29	0.06	0.00	0.01	0.01	0.13	0.00	0.18	0.06
Tangantangan, *Leucaena leucocephala* (leaves)	2.45	0.00	0.00	0.01	0.01	0.43	0.01	0.18	0.25
Tangantangan, *Leucaena leucocephala* (young seed pods)	0.86	0.05	0.00	0.00	0.00	0.19	0.00	0.20	0.14
Moringa, *Moringa oleifera* (leaves)	2.27	0.00	0.00	0.00	0.01	0.30	0.00	0.30	0.43
Moringa, *Moringa oleifera* (young seed pods)	0.27	0.00	0.00	0.00	0.02	0.17	0.00	0.40	0.44
Commercially Available:									
Cracked corns (*Zea mays*)	0.02	0.01	0.00	0.00	0.00	0.15	0.00	0.32	0.00
Lime	29.74	0.03	0.00	0.04	0.00	1.04	0.00	0.02	0.93

z Mineral contents of fish scrap were not determined.

STUDY 2: Feeding Trials

Ecofeed Trial 1

Materials and Methods

Recipe of Ecofeed A:
Based on the results from the nutrient analyses and availability of resources, the recipes for making layer feeds (Ecofeeds) were created for the feeding experiments. The 'Ecofeed A' was made using four food wastes including brewer's spent grain, kitchen wastes from restaurant M and C, and minced fish scraps and bones. From the farm, fruits of breadfruit and papaya, and leaves from leucaena (tangantangan) were added. Cracked corn was the main energy source of the diet and lime was added to supplement Ca to the feed. Sodium polyphosphate was added for its antimicrobial activity as a dietary supplement. Proportions of individual ingredients in 'Ecofeed A' are shown in **Table 3**. The feed was made using an animal feed making machine (Minori Sangyo, Japan) (**Fig. 10** and **Fig. 11**), and **Table 4** shows metabolizable energy, crude protein, calcium and phosphorus analysis of Ecofeed A and a commercial feed. Those feeds were stored in a container with a lid and were placed in a dark air-conditioned storage room until use.

Table 3. Type and amount of ingredients used in Ecofeed A.

Ingredient	Amount (g/kg)	%
Food Waste collected:		
Brewer's spent grain	225.4	22.5
Kitchen waste (Restaurant M)	220.7	22.1
Kitchen waste (Restaurant C)	62.4	6.2
Fish bones and scrap	11.7	1.2
From Farm:		
Breadfruit, *Artocarpus altilis* (fruit)	129.1	12.9
Tangantangan, *Leucaena leucocephala* (leaves)	92.9	9.3
Papaya, *Carica papaya* (fruits)	28.1	2.8
Commercially Available:		
Cracked corns (*Zea mays*)	191.8	19.2
Lime	35.9	3.6
Sodium polyphosphate	2.0	0.2
	Total	100.0

Fig. 10. Cooking the main part of Ecofeed including food waste, cracked corn, fruits of papaya and breadfruits, and tagantagan leaves.

Fig. 11. Finished product of Ecofeed.

Table 4. Metabolizable energy (ME), crude protein (CP), calcium (Ca) and phosphorus (P) analysis of Ecofeed A and a commercial feed

Feed	ME (MJ/kg)	CP (%)	Ca (%)	P (%)
Ecofeed A	11.1	13.8	2.98	0.229
Commercial feed[z] (tested in this study)	11.7	22.6	4.58	nd[x]
Commercial feed (shown on the label)	na[y]	20.0	4.0-5.0	>0.5

[z] Hawaiian Grain 20% Laying Pellets (Land O'lakes Purina Feed LLC.)
[y] na = not available on the label.
[x] nd = not determined in the study.

Feeding Procedure:
Rhode Island Red hybrid ISA Brown (*Gallus gallus domestica*) was used for the experiment. One-day old chicks were obtained from Hawaii and were raised by feeding a commercial grower feed until about 20 weeks old when they started to lay eggs. Then commercial layer feed was provided until the experiment began. The chickens were raised in a cage-free poultry house giving a free-range area during daytime with continuous water supply until the trials began. The first feeding trial was conducted using forty 52-week old layers divided into a group of five birds in a 3ft x 3ft x 4ft (90cm x 90cm x 120cm) cage (**Fig 12**). The trial was conducted for 11 days from Oct. 8 to Oct. 19, 2016 to compare a commercial feed and the 'Ecofeed A' for feed intake, egg production, and the bird weight. Each treatment was replicated four times. Feeds (140 g/bird) were given at 10 AM every day and water were provided daily. The feed intake was calculated by subtracting the feed remaining in the feeders from the amount of feed added to the feeders. Body weight of hens was recorded at the beginning and the end of the experiment.

Fig. 12.The first feeding trial set-up.

Results and Discussion

<u>Effects of 'Ecofeed A'</u>:
Feed intake was very poor for hens fed with 'Ecofeed A' throughout the duration of the experiment, compared to the commercial feed cages (**Fig. 13**). The average feed intake for the ecofeed and commercial feed was 54g/hen/day and 127g/hen/day, respectively. The effects of feed intake appeared on the 6th day of the experiment as egg production from the ecofeed hens was reduced. During Oct. 8 to Oct. 12, the average number of egg production from both treatments was 0.47 egg/hen/day. However, from Oct. 13 to Oct. 19, nearly no eggs were produced by hens fed with the ecofeed, while hens fed with the commercial feed maintained the same number of daily egg production (**Fig. 14**). The body weight was also decreased in hens fed with the ecofeed, while the body weight of hens fed with the commercial feed remained the same around 4 kg (**Fig. 15**).

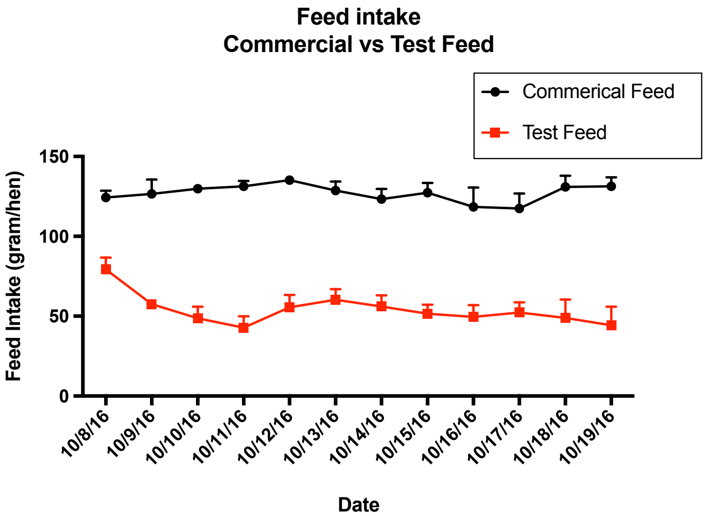


Fig. 13. Feed intake during the trail (n=4)

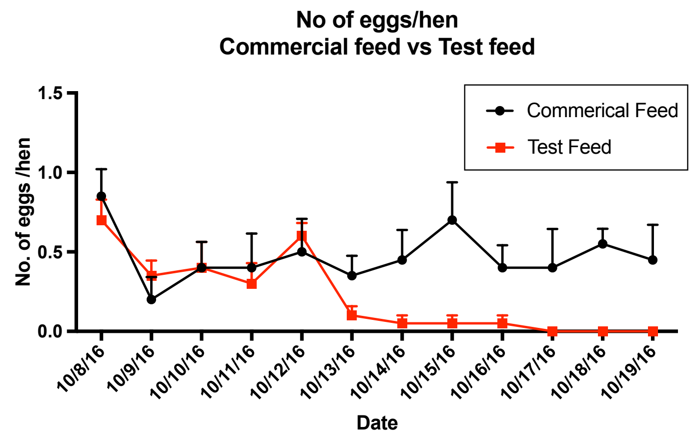


Fig. 14. Number of egg production/hen during the trial (n=4)

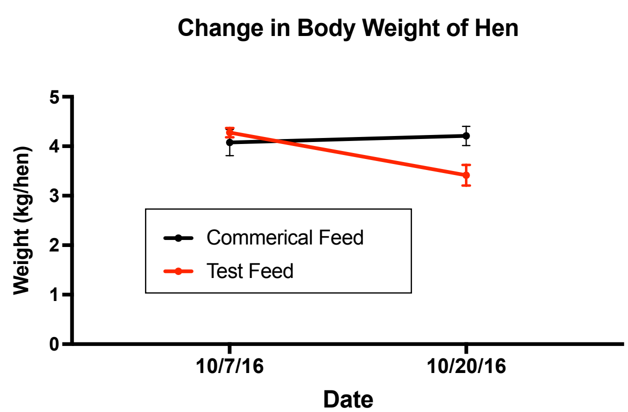


Fig. 15. Loss of body weight of layers fed with Test feed (100% Ecofeed A) compared to Control fed with a commercial feed. The graph is showing the mean and standard error of mean on before (10/7/16) and on the last day (10/20/16) of the trial (n=20).

Ecofeed Trial 2 and Trial 3

Materials and Methods

Recipe of Ecofeed B and Feed Treatment:
For Trial 2 and Trial 3, 'Ecofeed B' was constructed by modifying ingredients of 'Ecofeed A.' **Table 5** presents the ingredients in 'Ecofeed B' and three treatments tested in both trials. 'Ecofeed B' contained three food wastes, including spent grain, restaurant kitchen waste, and fish/bone scraps, as the source of crude proteins (**Table 1**). The commercial cracked corn and vegetable oil were added as a ME source. Dried leaves from two multipurpose trees (*Leucaena leucocephala* and *Moringa oleifera*) from a farm were also used as the source of fibers and minerals such as Ca, Mg, and Zn (**Table 2**). To increase palatability, honey and salt were also added with lime as an additional source of Ca to 'Ecofeed B.' Each test feed was stored in a container with a lid and placed in a dark air-conditioned storage room.

Three feed treatments were tested in Trial 2 and Trial 3 including Ecofeed 25%, Ecofeed 50% and control with 100% commercial layer feed. The ingredients, the metabolizable energy, and chemical analysis of the three feed treatments are shown in **Table 5**.

Table 5. The amount of ingredient (% dry matter) of three feed treatments tested in Trial 2 and Trial 3.

Ingredients	Treatment		
	Control	Ecofeed 25%	Ecofeed 50%
A. Commercial layer feed	**100%**	**75%**	**50%**
B. **Ecofeed B**:	**0%**	**25%**	**50%**
Ecofeed B Ingredients:			
Food waste and for crude protein:			
Spent grains		*5.95*	*11.91*
Food Waste (restaurant C)		*2.48*	*4.96*
Fish bones		*2.48*	*4.96*
For energy sources (ME TDN):			
Cracked corn (commercial)		*8.33*	*16.66*
Vegetable oil		*1.24*	*2.48*
For fiber and minerals:			
Leucaena leucocephala leaves		*2.23*	*4.46*
Moringa oleifera leaves		*0.25*	*0.50*
For minerals and increase in palatability:			
Lime		*1.79*	*3.57*
Honey		*0.19*	*0.39*
Salt		*0.05*	*0.11*
Total	100%	100%	100%
Metabolizable energy and chemical analysis:			
Crude Protein (%)	22.59	21.32	20.06
ME (Mcal/kg)	2.79	2.76	2.74
Calcium (%)	4.59	4.25	3.92
Phosphorus (%)	0.00	0.11	0.23

<u>Feeding Procedure and Data Collection</u>:

For Trial 2, sixty 38-week old Rhode Island Red hybrid layers were divided into three groups with four replicate flocks of five hens each. The experimental set-up was similar to Trial 1 and are shown in **Figs. 16-18**. Each cage had a feeder, an automatic waterer, a nest box and porches. Additionally, one 15-gallon plastic pot bedding with ironwood (*Casuarina equisetifolia*) needles was added to each cage as an alternative egg laying nest.

Fig. 16. Feeding trial set-up in Trail 2 and Trial 3.

Fig. 17. Cages were elevated with a mesh wire floor. A metal sheet was placed below the mesh floor to collect manure.

Fig. 18. A black pot with brown needles of ironwood was provided to lay eggs in addition to a nest box (not shown in this photo).

Trial 2 was conducted from December 27, 2016 to January 9, 2017 (14 days). Feeds (140 g/bird) were given at 10 AM and water was provided daily. The amount of feed intake, the number of egg production per cage, and the weight of eggs were recorded daily. Feed intake was calculated by subtracting the feed remaining in the feeders from the amount of feed added to the feeders, using the equation in the following box:

[The feed intake (g)] =
[the amount of feed in a feeder (g)] - [the amount of feed remaining in a feeder (g)]

Egg Productivity (%):

The egg productivity was determined by the number of eggs collected divided by the number of hens per cage per day. Daily egg mass (g) was determined by the average weight of eggs multiplied by the egg productivity (%) on the day of egg collection. Finally, the feed conversion was determined by the equation of the daily weight of the feed intake (kg) divided by the daily egg mass (kg). This feed conversion ratio indicated the amount of feed consumed to produce one kg of eggs, showing the efficiency of a test feed for egg production. For statistical analyses of daily feed intake (g), the egg productivity (%), daily egg mass (g) and feed conversation ratio were done using data collected for three days, January 7, 8, and 9.

Digestibility:

The digestibility of macronutrients and ME are other indexes to show efficiency of test feeds. The digestibility was determined by comparison of crude protein (CP), crude lipids (EE), crude ash (CA), crude fiber (CF), and nitrogen-free extract (NFE) in a test feed prior to providing to hens at 10 AM and the amount of those macronutrients recovered from bird manure on the following morning after 24 hours. A metal sheet was placed at the bottom of each cage to collect manure each day on January 8, 9, and 10. The dry matter content (DM), CP, EE, CA, CF, and NFE were determined according to the method by Abe (2001). The digestibility of each macronutrient was calculated by the equation of the AIA (acidified insoluble ash). Index is shown in the box:

$$\boxed{\begin{aligned}&\text{Digestibility of macronutrients (\%)}\\ &= \left(\frac{\text{AIA (\%) in a test feed}}{\text{AIA (\%) in manure}}\right) \text{x} \left(\frac{\text{Macronutrient (\%) in manure}}{\text{Macronutrient (\%) in the test feed}}\right)\end{aligned}}$$

Acidified Insoluble Ash (AIA):

To determine AIA, 5g of dry feed in 50 mL beaker was placed in a muffle furnace at 550°C for 5 hours until they became ash. For manure, 3g of sample was used. Ash in the beaker was transferred to a desiccator to cool them down for about 1 hour. After acid digestion with 30 mL of 4N HCl for 10 minutes, the sample solution was filtered using an ash-free filter paper. Remaining insoluble ash was then dried at 70°C for 8 hours and was placed in a muffle furnace at 550°C to determine AIA (%) for feed and manure.

Metabolizable Energy (ME):

The ME was calculated according to the methods described by Palie (2012) and Boisen (1997) from the value of the enzyme digestibility of organic matter (EDOM). The procedure was also described above in the materials and method of Study 1 in this paper.

In Trial 3, the feed experiment was repeated from February 13 to 24, 2017 (14 days) using the 45-week old Rhode Island Red hybrid layers and with the exact same experimental set-up. Sixty hens were divided into three groups with four replicate flocks of five hens each. The same feeding schedule described in Trial 2 was used. Daily feed intake and egg production were compared.

Results and Discussion

<u>Body Weight</u>:

The average body weight of hens at the initial and the final day of the experiment as well as the daily weight gain in Trial 2 are shown in **Table 6**. All hens increased in their body weight at the end of the trial.

<u>Feed Intake and Egg Productivity</u>:

The results of daily feed intake and egg productivity (%) of three feed treatments are shown in **Figs. 19 and 20**, respectively. We assumed that the first 4-5 days from the beginning of the experiment was a conditioning period when layers were adjusting to a new environment.

Reduction of feed intake was observed for the treatment of Ecofeed 50% especially toward the end of the experiment while the feed intake of Ecofeed 25% remained about the same as Control (Fig.19). Although feed intake with the treatment of Ecofeed 50% was the lowest, egg productivity (%) was compatible with Ecofeed 25% treatment and Control (Fig. 20).

Table 6. The initial and final body weight and the average daily weight gain of hens in Trial 2.[z]

Feed Treatment	Initial body weight (kg/hen)	Final body weight (kg/hen)	Daily weight gain (g/hen)
Control	1.69	1.85	11.7
Ecofeed 25%	1.70	1.94	16.9
Ecofeed 50%	1.68	1.82	9.9

[z] The weight and daily weight gain were calculated by weighing a group of 5 birds of cage each day. The average weight of hen is shown.

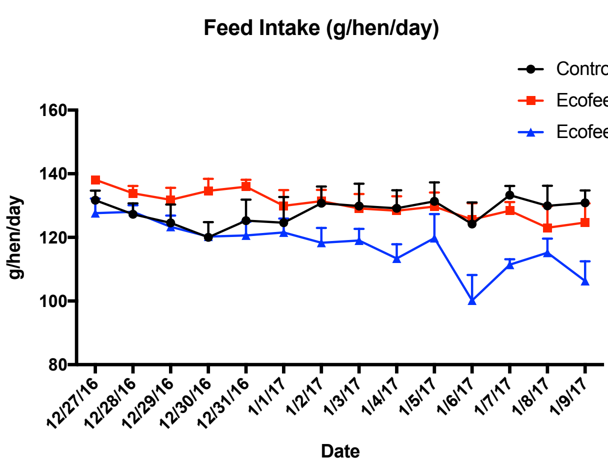


Fig. 19. Feed intake in the Trial 2.

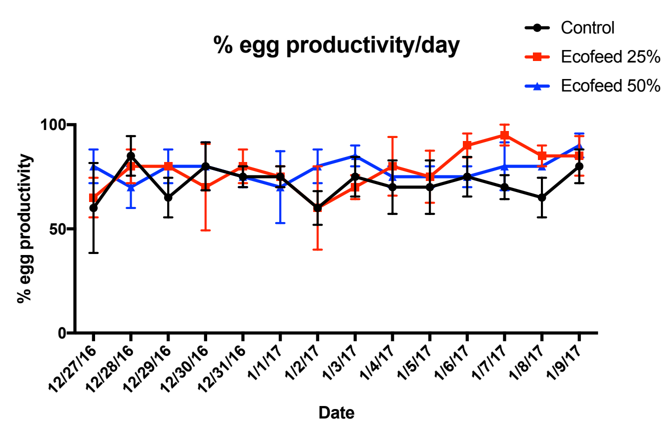


Fig. 20. Egg productivity (%) in the Trial 2.

Feed Conversion Ratio (FCR):

Table 7 shows the results of feed intake (g/day/hen), egg productivity (%), daily egg mass (g), and feed conversion ratio (kg feed/kg egg) during the last three days of the experiment from Jan. 8 to 10, 2017 in Trial 2. Feed intake of Control and Ecofeed 25% was higher than that of Ecofeed 50%, however both treatments of Ecofeed 25% and Ecofeed 50% had higher egg productivity and daily egg mass. Feed conversion ratio (FCR) was determined by the ratio of the feed weight intake over the average weight of an egg. **Lower FCR indicates higher efficiency of feed to be converted to the weight of egg**. Trial 2 found that FCR was lower with the two tested feeds, Ecofeed 25% and Ecofeed 50% than Control (Table 7), suggesting that the two Ecofeed treatments were efficiently converted to egg production compared to control.

Table 7. Comparisons of feed intake, egg productivity (%), daily egg mass and feed conversion ratio of feed treatments (Mean±SD) in **Trial 2** [z].

Feed Treatment	Feed intake (g/day/hen)	Egg productivity (%)	Daily egg mass (g)	Feed conversion ratio (kg feed/kg egg)
Control	131.4±8.6 a [y]	71.7±13.7 b	43.8±4.1 b	3.17±0.37 b
Ecofeed 25%	125.4±10.2 a	88.3±8.4 a	55.5±2.1 a	2.30±0.13 a
Ecofeed 50%	111.0±1.9 b	83.3±11.5 a	50.7±2.3 a	2.23±0.17 a

[Z] Treatment mean was calculated as the average from three days of data (1/8-1/10/17) for each treatment (n=4).
[y] Same letter after the treatment mean and standard deviation within a column is not significantly different (p<0.05).

Digestibility of Feeds:

The digestibility of DM, CP, EE, CF, and NFE, and ME is another indicator of the efficiency of test feeds to be converted to egg production. The digestibility was determined by comparison of amounts of DM and nutrients (CP, EE, CA, CF and NFE) in feed with those in manure in this study. **Table 8** shows the amount of **DM and nutrients in feeds**, Ecofeed B (100%), 'Control,' and the two test feeds. The Ecofeed (100%) had DM(94.5%), CP(13.6%), EE(11.1%), CA(11.6%), CF(4.9%), and NFE(58.7%). The 'Control' had DM(91.0%), CP(22.6%), EE(5.6%), CA(15.4%), CF(3.3%), and NFE(53.0%). The contents of DM and nutrients in 'Ecofeed 25%' and 'Ecofeed 50%' were estimated based on the values of Ecofeed B (100%) and 'Control.' **Table 9** shows the **DM and nutrients remained in manure** collected from hens fed with test feeds and control. The DM and CF contents in manure were similar among treatments, ranging 25.4-27.3% and 11.0-21.1%, respectively. CP and CA were higher in control than the two test feeds. CP of 'Control' was 33.0%, followed by 'Ecofeed 25%' (28.8%) and 'Ecofeed 50%' (27.5%). CA of 'Control' was 28.6%, followed by 'Ecofeed 25%' (26.9%) and then 'Ecofeed50' (22.2%). On the other hand, EE and NFE were highest with 'Ecofeed50' having 5.3% (EE) and 32.7% (NFE). 'Control' had the lowest with 2.1% EE and 25.4% NFE.

Table 8. The dry matter (DM) in **feed** and the nutrient components on the base of DM including crude protein (CP), ether extract crude lipids (EE), crude ash (CA), crude fibers (CF), and nitrogen-free extract (NFE) of Ecofeed B, and three feed treatments: commercial feed (Control), Ecofeed 25%, and Ecofeed 50% in **Trial 2.**						
Feed treatment	DM of feed (%)	Nutrient components (on the base of DM) in feed (%)				
		CP	EE	CA	CF	NFE
Ecofeed B (100%)	94.5	13.7	11.1	11.6	4.9	58.7
Feeds Treatment:						
Control	91.0	22.6	5.8	15.4	3.3	53.0
Ecofeed 25%[z]	91.9	20.4	7.2	14.4	3.7	54.4
Ecofeed 50%[z]	92.7	18.2	8.5	13.5	4.1	55.8

[z]Amounts of nutrients of two treatments, Ecofeed 25% and Ecofeed 50% are estimated by the nutrient values of control (commercial feed) and Ecofeed B. (100%).

Table 9. The dry matter (DM) of **manure**, and the nutrient components in manure on the DM base including crude protein (CP), ether extract crude lipids (EE), crude ash (CA), crude fibers (CF), and nitrogen-free extract (NFE) of three feed treatments: commercial feed (Control), Ecofeed 25%, and Ecofeed 50%**,** showing Mean±SD. Manure from each cage was collected for three days on January 8, 9 and 10 and were compiled as one sample for each cage (n=4).

Feed treatment	DM of manure (%)	Nutrient components on the base of DM remained in manure (%)				
		CP	EE	CA	CF	NFE
Control	25.8±0.5	33.0±1.4 a[z]	2.1±0.2 c	28.6±0.7 a	11.0±0.9	25.4±2.1 b
Ecofeed 25%	25.4±1.0	28.8±0.9 b	3.3±0.3 b	26.9±0.3 b	11.5±0.3	28.5±2.9 ab
Ecofeed 50%	27.3±4.3	27.5±0.8 b	5.3±0.3 a	22.2±0.8 c	12.1±0.4	32.7±1.2 a

[z] Same letter after the value of Mean ±SD within each column indicates that there are no significant differences among treatments (p<0.05).

Table 10 indicates the digestibility (%) of DM and nutrient components (CP, EE, CA, CF and NFE) and ME. The digestibility of DM, CP, EE, CA, CF and NFE was the highest with 'Control,' followed by 'Ecofeed 25%' and then 'Ecofeed 50%.' Statistically, the digestibility of DM was different between 'Control' and 'Ecofeed 50%' (p<0.05). The digestibility of CP and NFE was the same with 'Control' and 'Ecofeed 25%' and higher than that of 'Ecofeed 50%' (p<0.05). The EE was the highest with 'Control,' followed by 'Ecofeed 25%', and then by 'Ecofeed 50%' (p<0.05). CF did not show any difference among treatments statistically. ME was the same with 'Control' and 'Ecofeed 25%' which was higher than that of 'Ecofeed 50%.'

The digestibility of DM, CP, EE, and NFE was higher with a commercial layer feed ('Control') than the two test feeds. This result confirmed that nutrients in the commercial feeds were digested more. However, the egg productivity (%) and the daily egg mass were greater with 'Ecofeed 25%' and 'Ecofeed 50%.'than those of commercial feed (**Table 7**). This indicated that nutrients in ecofeed treatments were utilized efficiently in egg production.

Table 10. Digestibility (%) of dry matter (DM), crude protein (CP), ether extract crude lipids (EE), crude fiber (CF), and nitrogen-free extract (NFE), and metabolizable energy (ME) of feed treatments (n=4).

Feed Treatment	Digestibility (%)					ME (Mcal/kg)
	DM	CP	EE	CF	NFE	
Control	94.6±1.1 a[z]	72.1±5.4 a	93.1±1.4 a	37.4±12.2	90.9±1.6 a	2.99±0.10 a
Ecofeed 25%	93.9±0.4 ab	68.8±2.5 a	84.4±1.9 b	31.0±5.3	88.4±1.5 a	2.91±0.06 a
Ecofeed 50%	92.2±1.1 b	59.8±1.8 b	79.9±0.7 c	22.0±2.8	84.5±0.6 b	2.71±0.03 b

[z] Same letters after the value of Mean ±SD within each column indicate that there are no significant differences among treatments (p<0.05).

Feed Trial: Trial 3

Using the same experimental set up and methods, Trial 3 was conducted to confirm the results of feed intake and egg productivity found in Trial 2. The results of the two trials were very similar. **Fig. 21** presents the amount of feed intake of the three feed treatment for 10 days from 2/13/27 to 2/24/17. **Fig. 22** shows the % egg productivity. The feed intake was greater with 'Control' than those of 'Ecofeed 25%' and 'Ecofeed 50%' test feeds. Yet, egg productivity (%) was very similar among the three feed types during the last five days of the experiment. **Fig. 23** illustrates that layers consumed less 'Ecofeed 25%' and Ecofeed 50%' than birds fed 'Control' feed, however, egg productivity did not show any difference due to the type of feeds. The results suggest that 'Ecofeed' can be used as a supplement to commercial layer feed without losing egg production.

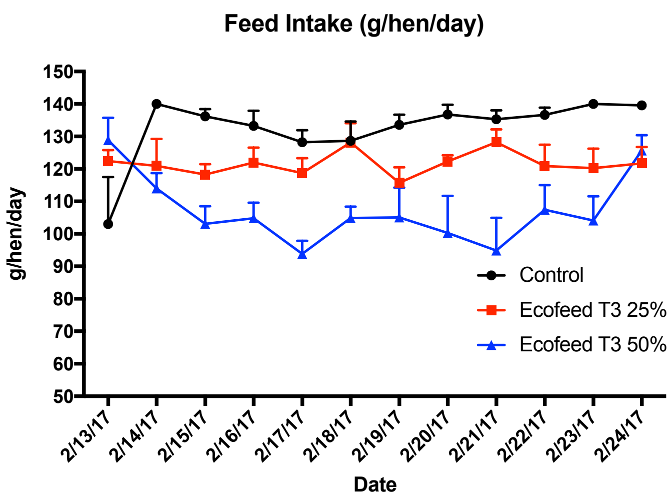


Fig. 21. Feed intake by hens in the Trial 3.

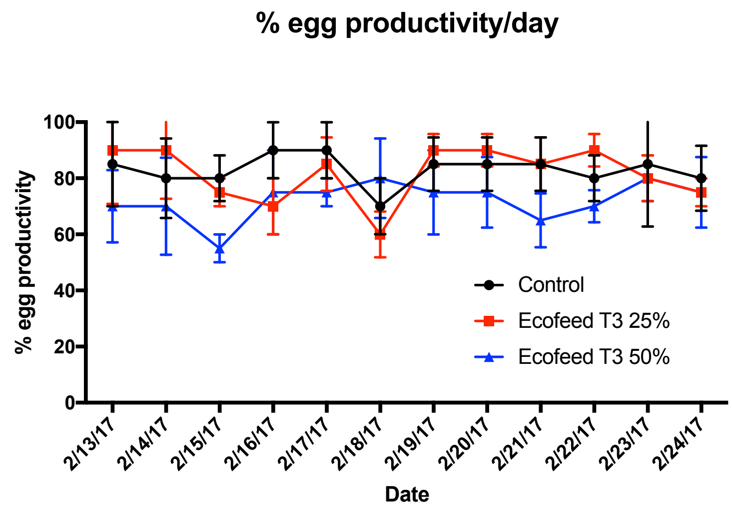


Fig. 22. Egg productivity (%) in the Trial 3.

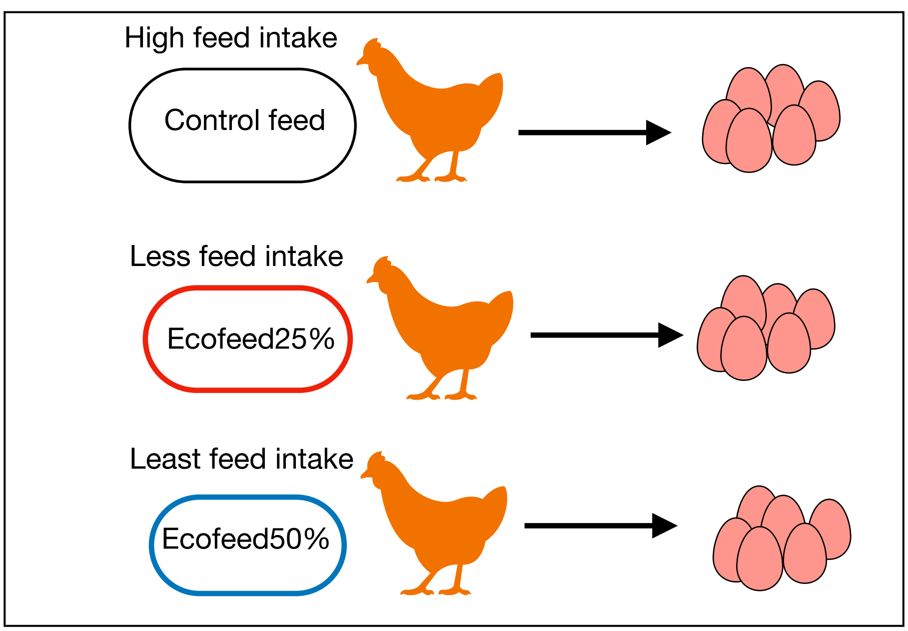


Fig. 23. Illustrating the similar egg productivity (%) by layers regardless with feed intake of 'Control' and less intake of test feeds in the Trial 3.

STUDY 3:

Effects of 'Ecofeed' on Egg Quality

Materials and Methods

Egg quality was studied for the egg weight, shell weight, shell thickness, yolk weight, albumen weight, and Haugh unit. Twenty eggs of each treatment were examined in Trial 2 and 15 eggs for Trial 3. The thickness of individual eggshells were measured with a caliper on three pieces of eggshell. **Haugh unit** was calculated from the records of albumen height (**Fig. 24**) and egg weight using the following formula (Nazok et al., 2010):

$$\text{Haugh Unit (HU)} = 100 \log(H - 1.7W^{0.37} + 7.56)$$

where H = the height of albumen (mm)
and W = the whole egg weight (g)

After the eggs were broken on a flat surface, the albumen and yolk were separated for measurement of their weight (**Fig. 25**). The **color of yolk** was determined using a colorimeter (Chroma Meter CR-200, Minolta, Inc.) to determine **L***, **a*** and **b***. **Chroma** was determined by the equation:

$$\text{Chroma (C)} = \text{SQRT}[(a^*)^2 + (b^*)^2].$$

In Trial 3, about ten grams of freeze-dried powered egg yolk from each treatment were sent to a commercial nutrition laboratory (Craft Technologies, Inc., North Carolina) to determine **carotenoid** contents by HPLC analysis.

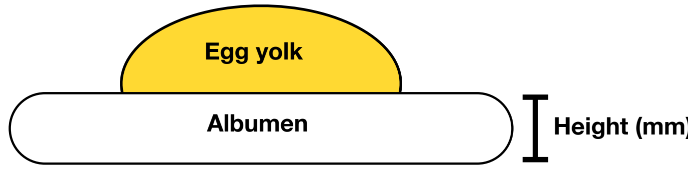


Fig. 24. Illustration of egg yolk and albumen, indicating the height of albumen.

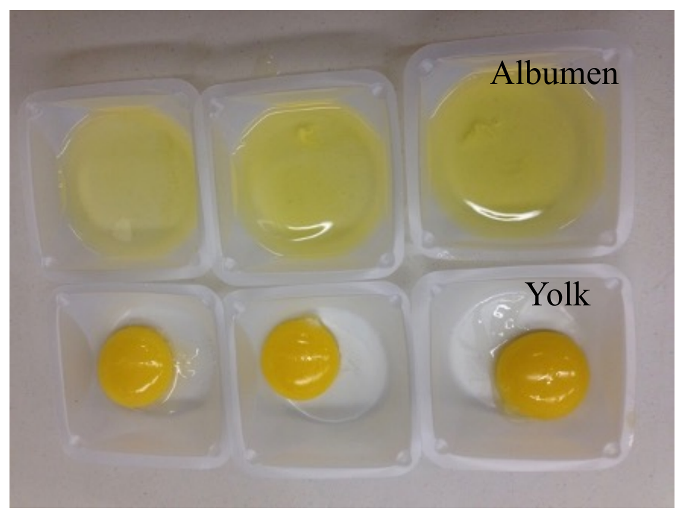


Fig. 25. Yolk and albumen of an egg were separated to measure their weights.

Results and Discussion

Egg Quality:

Table 11 and 12 show results of egg quality examined in Trial 2 and Trial 3, respectively. For both trials, the egg weight, shell weight, yolk weight, albumen weight, and Haugh Unit were not different among feed treatments, except there were thicker eggshells from Ecofeed 25% in Trial 3 ($p<0.05$). The study shows that layers fed with 25% and 50% local Ecofeecd can produce similar quality of eggs produced by those fed with the commercial feed. Two repeated experiments confirmed results with egg quality that eggs produced by test feeds incorporated with 25% and 50% ecofeed produced comparable quality of eggs as a commercial feed (Control). A slightly higher number in shell thickness and Haugh Unit from test feeds than control in both trials indicated that local ingredients in test feeds may contribute to improving the quality of eggs.

Table 11. Egg quality as the results of feed treatments, including egg weight, shell weight, shell thickness, yolk weight, albumen weight and Haugh unit in **Trial 2**, showing means and the standard error (n=20).

Feed treatment	Egg weight (g)	Shell weight (g)	Shell thickness (mm)	Yolk weight (g)	Albumen weight (g)	Haugh Unit
Control	61.0± 0.06	7.7± 0.06	0.55± 0.22	14.7± 0.09	37.5± 0.08	80.1± 0.11
Ecofeed 25%	62.8± 0.05	7.6± 0.08	0.64± 0.31	14.4± 0.13	39.2± 0.07	82.3± 0.12
Ecofeed 50%	60.9± 0.11	7.8± 0.10	0.65± 0.20	14.2± 0.10	37.5± 0.13	85.4± 0.08

Table 12. Egg quality as the results of feed treatments, including egg weight, shell weight, shell thickness, yolk weight, albumen weight and Haugh unit in **Trial 3**, showing means and standard error (n=15).

Feed treatment	Egg weight (g)	Shell weight (g)	Shell thickness (mm)	Yolk weight (g)	Albumen weight (g)	Haugh Unit
Control	63.4± 1.09	7.6± 0.20	0.51± 0.03 b[z]	16.1± 0.54	39.9± 0.91	78.5± 1.89
Ecofeed 25%	62.3± 1.13	7.6± 0.20	0.64± 0.04 a	15.6± 0.56	37.8± 0.84	79.9± 1.96
Ecofeed 50%	63.4± 1.05	7.7± 0.19	0.57± 0.03 ab	15.2± 0.52	40.0± 0.78	81.4± 1.83

[z] The same letter after mean and standard error indicates that the mean values of treatment are not significantly different ($p<0.05$).

<u>Yolk Color:</u>

Distinct difference was observed in **yolk color**. Yolk color was darker with both Ecofeed 50% and Ecofeed 25% than control (**Fig. 26**). The colorimeter readings from both Trial 2 (**Table 13**) and Trial 3 (**Table 14**) showed that Luminance (*L) was higher with Control than Ecofeed 25% and Ecofeed 50% indicating that egg yolk from Control was lighter in color. Values a* and b* also suggested that yolk color of two test feeds had higher hue of red and yellow, respectively, than that of Control. Chroma or color saturation compared in Trial 3 indicated that both Ecofeed 25% and Ecoffed 50% had higher value than control (p<0.05).

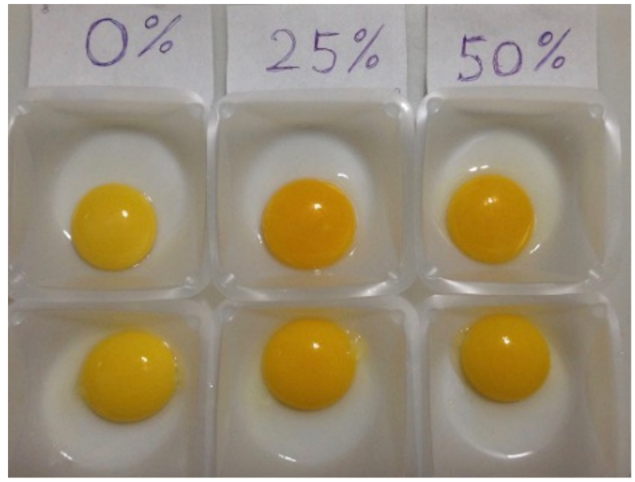


Fig. 26. Visual differences of yolk color: Control (0% of ecofeed) is lighter yellow compared to darker yolk color of two ecofeed treatments (25% and 50%).

Table 13. Colorimeter readings of egg yolk in **Trial 2** showing the means and standard error of Luminance (L*), value a* and value b* (n=20)

Feed Treatment	Luminance (***L**)	Value **a*** Red-Green	Value **b*** Blue-Yellow
Control	60.9± 0.03 a[z]	-6.3±0.14 c	29.9± 0.11 b
Ecofeed 25%	58.0± 0.05 b	-4.8± 0.19 b	36.2± 0.16 a
Ecofeed 50%	57.1± 0.05 b	-3.5± 0.29 a	36.3± 0.14 a

[z] The same letter after each number indicates that the mean values of treatment are not significantly different (p<0.05).

Table 14. Colorimeter readings of egg yolk in **Trial 3** showing the means and standard error of Luminance (L*), value a* and value b* and chroma (n=15)

Feed Treatment	Luminance (**L***)	Value **a*** Red-Green	Value **b*** Blue-Yellow	Chroma
Control	60.9± 0.64 a[z]	-5.95± 0.393 b	30.8± 1.13 b	4.9± 0.12 b
Ecofeed 25%	58.8± 0.67 ab	-4.18± 0.408 a	36.9± 1.18 a	5.7± 0.13 a
Ecofeed 50%	58.1± 0.62 b	-4.09± 0.380 a	37.9± 1.10 a	5.8± 0.12 a

[z] The same letter after each number indicates that the mean values of treatment are not significantly different (p<0.05).

Carotenoid in Yolk:

The total **carotenoid** content in egg yolk shown in **Fig 27** was highest with Ecofeed 25%, followed by Ecofeed 50% and the least for Control ($p<0.05$). The amounts of major carotenoid compounds detected in egg yolk of three feed treatments are shown in **Table 15.** Darker yolk of Ecofeed 25% and Ecofeed 50% might have been due to addition of *Leucaena leucocephala* and *Moringa oleifera* leaves (Lopes et al., 2014; Teteh et al., 2016). Darker yellow or orange color of egg yolk was due to higher concentrations of carotenoids in egg yolk.

Table 15. The amount of major carotenoid compounds detected in egg yolk of three feed treatments (n=3)

Feed treatment	trans-Lutein	trans-Zeaxanthin	cis-Lutein & Zeaxanthin	alpha-Cryptoxanthin	beta-Cryptoxanthin	trans-beta-Carotene	cis-beta-Carotene	Total beta-Carotene
	mcg/g	mcg/g	mcg/g	mcg/g	mcg/g	mcg/g	mcg/g	mcg/g
Control	5.63 b [z]	2.55 b	0.40 b	0.14 a	0.19 b	0.05 b	0.02 b	0.07 b
Ecofeed 25%	26.62 a	6.18 a	0.72 a	0.14 a	0.42 a	0.53 a	0.19 a	0.72 a
Ecofeed 50%	19.84 a	5.39 a	0.65 ab	0.16 a	0.35 a	0.34 ab	0.16 ab	0.50 ab

[z] The same letter after each number indicates that the mean values of treatments are not significantly different ($p<0.05$).

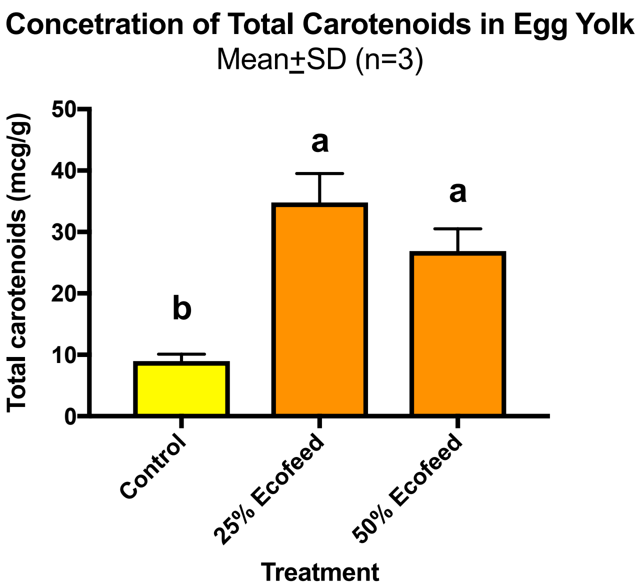


Fig. 27. Total carotenoid contents in egg yolk of Ecofeed 25%, Ecofeed 50%, and Control feed treatments in Trial 3. The same letter after each number indicates that the mean values of treatment are not significantly different ($p<0.05$).

SUMMARY

The study suggests that locally made poultry feedstock using food waste and other bio-resources can supplement commercial layer feeds with additional nutrition values.

The nutrient analyses of local materials showed that crude protein (CP), one of most important nutrients needed for layer feed, was most in fish scrap and less in kitchen waste from restaurants. Also, there were CP from leaves and young green seed pods of tangantangan (*Leucaena leucocephala*) and moringa (*Moringa oleifera*). Nutrients in kitchen wastes varied depending mainly on the types of food served at restaurants. The nutrient analyses of such food waste are essential when we construct feedstock to layers.

In the first feed experiment, Trial 1, feed intake of test feed, '100% Ecofeed A,' was very poor, and resulted in the reduction of egg production on the 6th day of the experiment and eventually nearly no egg production. The body weight was also decreased with feeding the '100% Ecofeed A.'

In Trial 2, with the improved recipe of 'Ecofeed B' and mixing 25% and 50% of this feed with commercial layer feed, both 'Ecofeed 25%' and 'Ecofeed 50%' had higher egg productivity and daily egg mass than Control (100% commercial feed). The feed conversion ratio (FCR) was lower with the two test feeds than 'Control.' This indicated that 'Ecofeed 25%' and 'Ecofeed 50%' had higher efficiency of feed being converted to the weight of egg than that of 100% commercial feed.

Egg quality was also examined including the egg weight, shell weight, yolk weight, albumen weight, and Haugh Unit. It was found that egg quality had no difference among feed treatments. However, yolk color was much darker with test feeds containing high concentrations of carotenoids. It appears that addition of green leaves in test feeds may be responsible for dark egg yolk with carotenoids.

The next step would be to explore cost-effective ways of collecting a large quantity of bio-waste to make poultry feeds containing good nutrients commercially. Another issue can be a long term storage of feedstock in our tropical humid environment. At the end of the study, we explored pelletizing feeds and vacuum packing feeds. **Fig. 28** shows examples of pelletized feeds and **Fig. 29** shows an example of vacuum sealed pelletized feeds for long term storage.

Fig. 28. Examples of pelletized local feeds.

Fig. 29. Vacuum sealed pelletized local feeds for long term storage.

REFERENCES

Abe, R. 2001. New animal nutrition test methods. Ishibashi, T (eds). Yokendo, Tokyo. pp. 455-466.

Boisen, S. and J.A. Fernandez. 1997. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. Animal Feed Science Technology. 68:277-286.

Duguies, M.V., Victor T. Artero, Jeff D. T. Barcinas. 2016. Poultry Production Guide for a 500 Layer Operation. CNAS, University of Guam. 2nd Revised, pp: 1-2.

Palić, D. D. Okanović, Psodorov, Natalija Džinić, Slobodan Lilić, Vladislav Zekić, Dragan Milić. 2012. Prediction of metabolizable energy of poultry feeds by estimating in vitro organic matter digestibility. African Journal of Biotechnology. 11: 7313-7317.

Losinio, L. 2013. Senator eyes zero food waste system for Guam. *Marianas Variety. 2013 April 29.* Retrieved from http://mvguam.com/local/news/29240-senator-eyes-zero-food-waste-system-for-guam.html.

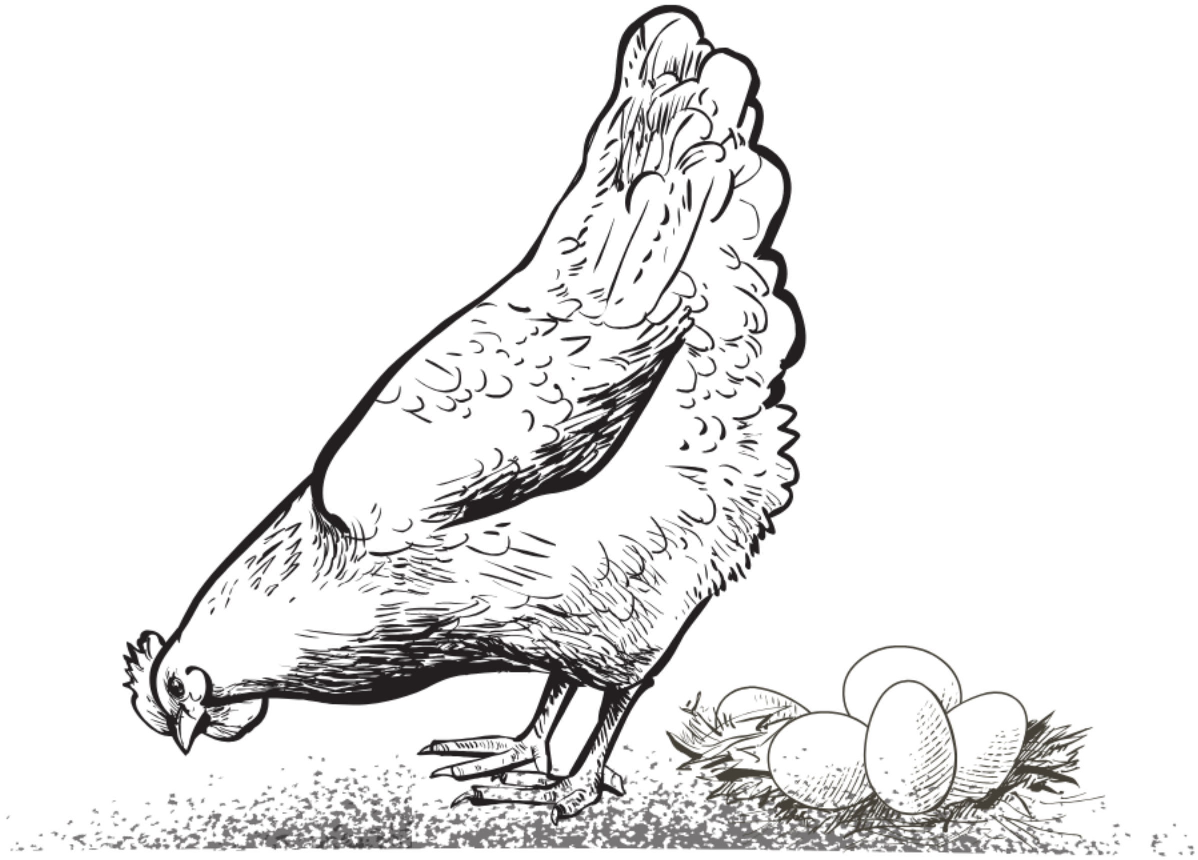
Pobocik, R.S., A. Trager, L. Morrell Monson. 2008. Dietary patterns and food choices of a population sample of adults on Guam. Asia Pac J Clin Nutr. 17:94-100.

Nazok, A. Mansour Rezaei & Hadi Sayyahzadeh. 2010. Effect of different levels of dried citrus pulp on performance, egg quality, and blood parameters of laying hens in early phase of production. Trop. Anim. Health Prod. 42:737–742 DOI 10.1007/s11250-009-9481

Isaac, R.A. 1998. AOAC Method 985.01. Metals and Other Elements in Plants. In: Official Methods of Analysis of AOAC International. 16th ed. P. Cunniff (ed.), Gaithersburg, Md: AOAC International.

Lopes, I.R.V., E.R. Freitas, G.A.J. Nascimento, Viana Neto, C.E.B. Cruz, N.M. e Braz. 2014. Inclusion of *Leucaena leucocephala* and *Clitoria ternatea* leaf hays into laying hen diet, Archivos de Zootecnia, 63(241): 183-190.

Teteh, A., M. Gbeassor, E. Decuypere, K. Tona. 2016. Effects of Moringa oleifera Leaf on Laying Rate, Egg Quality and Blood Parameters, International Journal of Poultry Science 15 (7): 277-282.



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