

# EFFECT OF DIETARY INCLUSION OF FERMENTED PINEAPPLE PEEL WASTE ON PERFORMANCE AND EGG YOLK CHOLESTEROL LEVEL OF LAYING HENS

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↳Supporting Information

**ABSTRACT:** The experiment was conducted to evaluate the effect of fermented-pineapple peel waste (FPPW) with indigenous microorganisms (IMO) solution from bamboo sprout on performance and egg yolk cholesterol of laying hen ration. In this experiment, a total number of 200 ISA Brown laying hens with 32 weeks old with 70% egg production and average egg weight 58.58 g/egg were used. A completely randomized design was used in this experiment. Five different levels of supplementation with FPPW (0, 5, 10, 15, and 20%) were used in ration, and each treatment was replicated four times. The measured parameters were daily feed consumption, daily egg production, egg weight, egg mass, feed conversion ratio (FCR), and egg yolk cholesterol. The results indicated that FPPW with IMO solution from bamboo sprout as much as 20% reduced egg yolk cholesterol significantly, and did not affect daily feed consumption, daily egg production, egg weight, egg mass, and FCR significantly. It has been concluded that FPPW could be used up to 20% in laying hens rations without disrupting performance (daily feed consumption, hen day egg production, egg weight, egg mass, and FCR), and could reduce egg yolk cholesterol as much as 16.86%.

**Keywords:** Bamboo, Laying hens, Performance, Pineapple peel, Waste.

## INTRODUCTION

Waste materials from agriculture products have the potential to be used as poultry ration ingredients to exploit the remaining nutritional content. Pineapple peel waste is one of the waste materials that has not yet been widely used as a poultry feed. Pineapple production in Indonesia is around 1.39 million tons/year (Pariona, 2018). Base on Indonesia Central Bureau of Statistics (2017 and 2018), the pineapple export from Indonesia was 8.024.662 kg and 13.362.430 kg, respectively. As much as 30% of pineapple peel is produced from one pineapple fruit's processing into edible products (Campos et al., 2020). The nutrients content profile of pineapple peel waste on dry matter basis were 93.79% dry matter, 5.76% crude protein, 24.00% crude fiber, 0.93% crude fat, 6.08% ash, 0.53% calcium, 0.25% phosphorous, and it contains 3699.8 kcal/kg gross energy (Mahata et al., 2016), and up to 38.3 mg/100g vitamin C (Ellong et al., 2015). Vitamin C is known to increase the feed consumption in poultry (Torki et al., 2014; Abudabos et al., 2019).

These waste feedstuffs can be utilized by poultry; however the limiting factor for using pineapple peel waste as poultry feed is that the high crude fiber makes it difficult to digest (Aruna, 2019). Therefore it needs to be processed to reduce crude fiber content before adding it to the poultry feed. A fermentation process with an indigenous microorganisms (IMO) solution derived from bamboo sprouts is one method to reduce the crude fiber content in pineapple peel waste (Adrizal et al., 2017). The *In vitro* testing of bamboo sprouts which had been cultivated on cellulase-producing medium (Carboxymethyl Cellulose or CMC agar plate) revealed the presence of *Bacillus thuringiensis*, *Bacillus aerius*, *Lactobacillus plantarum* and *Panus velutinus* (Mahata, 2019). When IMO microorganisms were used to ferment pineapple peel, the crude fibre has been found to be reduced by 17.16% leaving 12.85% water, 87.15% dry matter, 8.95% crude protein, 2.08% crude fat, 0.16% calcium, 0.38% phosphorus, and 1190.47 kcal/kg metabolic energy (Adrizal et al., 2017). This fermented pineapple peel waste (FPPW) can be used to replace up to 12% of broiler ration without interfering with carcass performance and organ development (Heryandi et al., 2018). Present experiment evaluated the effect of FPPW on performance and egg yolk cholesterol of laying hens.

## MATERIALS AND METHODS

### Laying hens and housing

In current experiment a 200 number of ISA Brown laying hens that aged 32 weeks were used that had 70% egg production, average egg weight 58.58 and body weight of 1.62 kg. The breed of laying hens was used was ISA Brown. It was purchased at the poultry shop in West Sumatra province, Indonesia. This research was conducted on an independent farm laying hens in West Sumatra, Indonesia. The adaptation period was carried out to adjust the laying hens with rations treated with fermented pineapple peel waste (FPPW) for one week (seven days). The cage used was a battery cage with

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as many as 200 cages. The light system was arranged appropriately with laying hens need in the night with lamp, and a long day the lamp is off.

#### Date of study

This research was conducted from 8th November 2018 to 2<sup>nd</sup> January 2019 for a period of eight weeks.

#### Ethical approval

The present study was approved by the Animal Ethics Committee of Andalas University in Padang, Indonesia (No. 708/KEP/FK/2019).

#### Vaccine and vitamins

The vaccine given was Medivac Coryza T Suspension through subcutaneous. The vitamin given was a top mix produced by the feed industry in Indonesia named Medion. top mix composition for each 10 kg contain: 12.000.000 IU vitamin A, 2.000.000 IU vitamin D3, 8.000 IU vitamin E, 2.000 mg vitamin K3, 2.000 mg vitamin B1, 5.000 mg vitamin B2, 500 mg vitamin B6, 12.000 µg vitamin B12, 25.000 mg vitamin C, 6.000 mg Calcium-D-pantothenate, 40.000 niacin, 10.000 mg cholin chloride, 30.000 mg methionine, 30.000 mg lysine, 120.000 mg manganese, 20.000 mg iron, 200 mg iodine, 100.000 mg zinc, 200 mg cobalt, 4.000 mg copper, 10.000 mg santonin (antioxidant), 1.300.000 mg growth promoter.

#### Experimental design and ration

The experiment was designed in a completely randomized design using a treatment ration containing five different percentages of FPPW (0, 5, 10, 15, and 20%), and each treatment was repeated four times. Rations containing 0% FPPW was control rations, and those containing 5, 10, 15, and 20% FPPW were treatment rations. Laying hens have placed as many as ten birds in the battery cage for each control and treatment ration and its replication.

The ration was self-prepared using Yellow corn, palm oil, rice bran, top mix, cargill concentrate, stone flour, and fermented pineapple peels waste, as shown in Table 1. These mixed rations were formulated to be iso-protein (16%) and iso-energy (2600 kcal/kg).

**Table 1 - Ration composition, nutrient content (%), and metabolizable energy (kcal/kg ration) of the experimental ration**

Experimental ration % (FPPW)	Feedstuffs (%)							
	Yellow corn	Concentrate for laying hens	Rice bran	FPPW	Palm oil	Top mix	Flour stone	Total
0	44.00	28.00	23.00	0.00	0.25	0.50	4.25	100.00
5	43.00	28.00	18.75	5.00	0.75	0.50	4.00	100.00
10	42.00	28.00	14.50	10.00	1.25	0.50	3.75	100.00
15	41.00	28.00	10.25	15.00	1.75	0.50	3.50	100.00
20	40.00	28.00	6.00	20.00	2.25	0.50	3.25	100.00

  

Experimental ration % (FPPW)	Calculated analysis							
	Crude protein (%)	Crude fiber (%)	Crude fat (%)	Calcium (%)	PA (%)	Methionine (%)	Lysin (%)	ME (kcal/kg)
0	16.48	5.80	3.93	3.47	0.26	0.15	0.22	2623.92
5	16.37	6.11	4.23	3.42	0.27	0.14	0.20	2623.77
10	16.27	6.41	4.53	3.37	0.28	0.13	0.17	2623.62
15	16.16	6.72	4.59	3.33	0.28	0.11	0.14	2623.47
20	16.05	7.02	5.12	3.28	0.29	0.10	0.12	2623.32

FPPW: Fermented pineapple peel waste; PA: Phosphor available; ME: Metabolizable energy.

#### Preparation of IMO solution from bamboo sprout

Chopped bamboo sprouts weighed as much as 1000 g and mix with 3L of rice water and 200g of palm sugar. Furthermore, stirred until homogeneous and incubated under anaerobic conditions for 15 days. The harvested bamboo sprouts IMO solution was separated between the liquid and the bamboo sprouts dregs, this liquid was the indigenous microorganisms.

#### Preparation of FPPW

Pineapple peel was obtained from pineapple home industry processing in Kampar Regency, Riau province. This province is the second-largest pineapple producer on the island of Sumatra, Indonesia. Pineapple peel was prepared by cleaned, and then they were weighed as much as 500 g and put in a fermentor. As much as 325 ml IMO solution was added and mixed until the mixture became homogeneous. Furthermore, it was incubated for one week. The fermentation process was stopped after incubation finished, dried under the sun, the milled becomes powder. Furthermore, it was ready to be used as poultry feed (Adrizal et al., 2017).

### The measured parameters

**Daily feed consumption** is calculated by the total number of rations given to laying hens for one week minus the number of rations left for one week, then divided by the number of days for one week (7 days).

The **hen day egg production** was measured by the method of [Cesari et al. \(2014\)](#) with the formula as follows:

$$\text{Hen day egg production} = \frac{\text{The number of eggs produced on the day of measurement}}{\text{The number of live laying hens on the day of measurement}} \times 100\%$$

The following formula measured egg weight:

$$\text{Egg weight} = \frac{\text{The weight of eggs produced in each unit}}{\text{The number of eggs produced in each unit}}$$

The **Egg mass** was calculated using the method of [Cesari et al. \(2014\)](#) with the following formula:

$$\text{Egg mass} = \text{hen day egg production (\%)} - \text{egg weight (g)}.$$

The **feed conversion ratio** was calculated using the method of [Kulshreshtha et al. \(2014\)](#) with the following formula:

$$\text{Feed conversion ratio} = \frac{\text{Ration consumed (kg)}}{\text{Egg production} \times \text{egg weight (kg)}}$$

**Egg yolk cholesterol** measurements were carried out by using spectrophotometry. After weighing 1 gram of egg sample and putting it in a centrifuge tube which contained 10 ml of acetone alcohol solution (1:1) and then warming was performed until the solution boiled. After cooling the solution, it was centrifuged at 3000 rpm for 15 minutes, the supernatant was taken and then evaporated on a boiling water bath until the residue remained, after that, dilution was carried out with chloroform. The reading performed at 680 nm, after the color turned green ( $\pm 5$  minutes). The calculation of cholesterol levels was calculated by comparing the absorbance with the cholesterol standard curve.

The equation was:  $Y = 2.354X + 0.005$

Y = Absorbance of cholesterol sample; X = level of cholesterol (mg/100ml)

### Statistical analysis

The research data were analyzed using analysis of variance for a Completely Randomized Design. The significance of differences between treatments were tested with Duncan's Multiple Range Test (DMRT), with a significant value at  $P < 0.05$  and  $P < 0.01$  ([Steel and Torrie, 1995](#)).

## RESULTS AND DISCUSSION

The results showed the inclusion of 0, 5, 10, 15 and 20% FPPW had no significant effect ( $P > 0.05$ ) on daily feed intake, hen day egg production, egg weight (Table 2). Over time, no difference in egg mass, and FCR from the five treatments were observed ( $P > 0.05$ ; Table 3). The use of FPPW as much as 0, 5, and 10% in rations had no significant effect ( $P > 0.05$ ) on egg yolk cholesterol, however the inclusion of 15 and 20% FPPW in ration had a highly significant ( $P < 0.01$ ) effect on lowering egg yolk cholesterol (Table 3).

Increasing the use of the FPPW up to 20% changed the color of ration the initially yellowish ration to brown as the amount of corn was reduced from 44% to 40%. This did not appear to have any effect on the palatability of the ration as the hens seemed to find the taste of FPPW attractive. Besides, high vitamin C content in FPPW is thought to trigger an increasing palatability of the ration. Pineapple is rich in Vitamin C containing 6.40 mg/100 ml ([Nweze et al., 2015](#)). Inclusion of 250 g/kg vitamin C in laying chicken ration has been found to increase the feed consumption by 0.75% [Torki et al. \(2014\)](#). Furthermore, [Abudabos et al. \(2019\)](#) stated the inclusion of vitamin C as much as 200 mg/L in broiler ration with environment's temperature is 32 °C, showed increasing the feed consumption of broiler up to 0.98%.

FPPW also contains a bromelain enzyme which has been shown to not interfere with feed consumption, reduce blood serum cholesterol, and liver cholesterol in laying hens ([Lien et al., 2012](#)). Replacing up to 12% of ration with FPPW has been shown to have no effect on the feed consumption ([Handika, 2017](#)). The average daily feed consumption of laying hens in this study ranged from 116.01 to 118.25 g/bird/d. These results are similar to those reported by [Yenice et al. \(2019\)](#) who also found the inclusion of bromelain enzymes in laying hen ration did not affect daily feed consumption which remained at 114 to 119 g/bird/d. Daily feed consumption affects hen day egg production, egg weight, and egg mass. In this study, the inclusion of the FPPW up to 20% had no effect on consumption so hen day egg production, egg weight, and egg mass also remained the same. Other determinants of egg production are ration nutrient content, age of livestock, protein level, production period, energy, fat, vitamins, mineral rations, and water ([Suprijatna, 2005](#); [Agustina and Purwanti, 2012](#)), which remained similar for all treatments. Nutrition contained in each treatment was equivalent as it was formulated to be iso-protein (16%) and iso-energy (2600 kcal/kg), and the age of all laying hens was 32 weeks. The results of this study are lower than reported by [Yenice et al. \(2019\)](#), who found the inclusion of bromelain enzymes in rations of laying hens did not affect hen day egg production which was 87.7 to 90%.

Inclusion of FPPW did not affect egg weight as it did not affect daily feed consumption or subsequent nutrient intake or any other factors that influence egg weight such as amino acid intake, genetic traits, age, weight gain, and drugs (Keshavarz, 2003; Hartono and Kurtini, 2015; Saputra et al., 2016). The average egg weight in this study ranged from 62.88 to 63.68 g/egg which are almost the same as those reported by Lien et al. (2012) who found the use of bromelain enzymes in laying hens rations did not disturb egg weight which was 64.87 to 66.03 g/egg. The inclusion of FPPW in the ration also did not affect egg mass. This is to be expected as egg mass is the product of egg weight and egg production. Ration that was composed of up to 20% of FPPW did not affect FCR due to the nutritional content of ration in each treatment being the same for all treatments. According to Andriyanto et al. (2015), one of the factors affecting FCR is nutrition quality. The average FCR in this study ranged from 2.65 to 2.83. The result of this study was higher than those reported by Yenice et al. (2019) who measured a FCR range from 1.97 to 2.01.

The use of FPPW had a significant ( $P < 0.05$ ) impact on egg yolk cholesterol (Table 3), decreasing the egg yolk cholesterol content to 16.86%. The decrease of egg yolk cholesterol in this study was due to the activity of the bromelain enzyme contained in the FPPW which was 0, 0.0016, 0.0032, 0.0048, 0.0064 U/mL for treatments A, B, C, D, and E, respectively. Bromelain enzyme is a sulfhydryl protease enzyme that can hydrolyze proteins into simple water soluble amino acids (Gautam et al., 2010). The biological effects of bromelain are related to their proteolytic activity (Mazorra-Manzano et al., 2018). The structure of the bromelain enzyme is resistant to stomach acid so up to 40% can be absorbed in the digestive tract of livestock (Lien et al., 2012). The cholesterol reducing mechanism of bromelain is not clearly understood. Cholesterol in the body comes from diet and the liver (Murray et al., 2000). Hu et al. (2020) reported that bromelain could inhibit the absorption of fatty acids and the secretion of Very Low-Density Lipoprotein (VLDL), further reported that giving bromelain as much as 20 mg/kg in rats fed a high-fat diet for 12 weeks can reduce glycerol levels and fat accumulation in the liver. Bromelain breaks down the plaque on the walls of blood vessels improving blood circulation (Kelly, 1996). Previous researchers reported that bromelain enzyme in laying hen ration can reduce both blood serum and liver cholesterol (Lien et al., 2012; Yenice et al., 2019).

**Table 2 - The average daily feed consumption, hen day egg production, and egg weight of laying hens fed FPPW**

Treatment (FPPW %)	Daily feed consumption (g/bird/d)	Hen day egg production (%)	Egg weight (g)
0	117.86 ± 0.89	70.92 ± 1.67	62.96 ± 2.75
5	116.01 ± 2.24	69.64 ± 4.26	63.24 ± 0.74
10	118.25 ± 1.07	65.66 ± 7.10	62.88 ± 1.95
15	117.17 ± 0.98	69.08 ± 6.80	62.95 ± 1.14
20	117.89 ± 0.47	66.28 ± 5.72	63.68 ± 0.30
SE	0.64	2.74	0.82
P-value	NS	NS	NS

FPPW= fermented pineapple peel waste, NS= non-significant

**Table 3 - The average egg mass, feed conversion ratio, and egg yolk cholesterol of laying hens fed FPPW**

Treatment (FPPW %)	Egg mass (g)	Feed conversion ratio	Egg yolk cholesterol (mg/100 g)
0	44.65 ± 2.35	2.65 ± 0.13	518.07 <sup>a</sup>
5	4.06 ± 2.36	2.66 ± 0.19	519.55 <sup>a</sup>
10	41.49 ± 3.57	2.88 ± 0.25	480.35 <sup>ab</sup>
15	43.24 ± 3.53	2.74 ± 0.27	468.02 <sup>bc</sup>
20	42.21 ± 3.61	2.83 ± 0.24	430.70 <sup>c</sup>
SE	1.57	0.11	14.81
P-value	NS	NS	$P < 0.01$

SE= Standard error, FPPW = fermented pineapple peel waste; <sup>a,b,c</sup> = Means in a column that are not followed by the same letter are significantly different at ( $P < 0.01$ ).

## CONCLUSION

Fermented pineapple peel waste could be used up to 20% in laying hens rations without disrupting performance (daily feed consumption, hen day egg production, egg weight, egg mass, and feed conversion ratio), and can reduce egg yolk cholesterol as much as 16.86%. At the level 20%, obtained the daily feed consumption was 117.89 g/bird/d, hen day egg production 66.28%, egg weight 63.68 g, egg mass 42.21 g, feed conversion ratio 2.83, and egg yolk cholesterol 430.70 mg/100 g.

## DECLARATIONS

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### Authors' contribution

Adrizal and Maria Endo Mahata participated in all stages of the research, namely the research design, the conduct of the experiment, sample analysis, data analysis, writing, and editing of articles. Nurul Hidayat participated in conducting the investigation, Yan Heryandi was responsible for data analysis. All authors participated in writing the article.

### Conflict of interests

All authors declared they have no conflict of interest concerning the work presented in this manuscript.

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