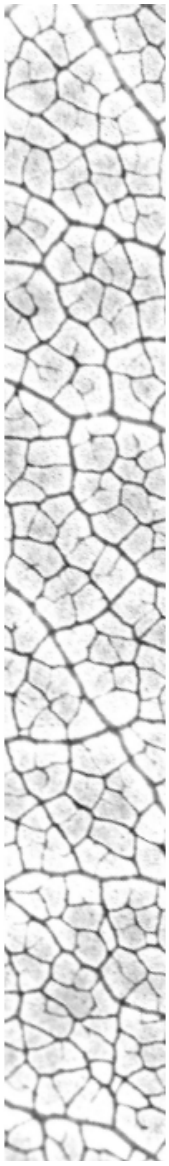

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Effect of walnut (*Canarium vulgare* L.) provisioning on white rat biology

Meitycorfrida Mailoa [1*], Tri D. Widyaningsih [2], Widya D.R. Putri [2], Harijono [2]

[1] Department of Agricultural Technology, Faculty of Agricultural, Pattimura University, Ambon 97233, Maluku, INDONESIA
[2] Department of Agricultural Technology, Faculty of Agricultural Technology, Brawijaya University, Malang 65145, East Java, INDONESIA
*Corresponding author: meitymailoa@gmail.com

Abstract
Several indicators can be used to discover whether a food is a source of high-quality protein. Protein Efficiency Ratio (PER) is a food protein quality measure defined by the protein's ability to generate growth of white rats. This study aimed to analyze the protein quality from fresh walnuts and dried walnuts *(Canarium vulgare* L.) as measured by the growth of white rats. Male Wistar strain white rats (*Rattus novergicus* Berkenhout) as the animal model. Three groups of newly weaned white rats (21 days old), which had been adapted for 4 days so they had the relatively average of weight (50.22 to 53.97 g) were used. Each group contained four individuals and they were fed different feeds, i.e., normal AIN coupled with 9% casein, normal AIN coupled with 1.8% fresh walnuts and normal AIN coupled with 1.8% dried roasted walnuts. Rat weights were measured every 2 days and feed consumption measured every day, for 28 days. Tukey's HSD Test was used to compare treatments. The results showed that there was a significant difference ($P < 0.05$) between the PER treatment of AIN feed coupled with dried roasted walnuts (PER: 2.58) compared with AIN coupled with fresh walnuts treatment (PER : 2.54) and AIN added with casein (PER : 2.52), but those three treatments showed a higher PER value than the standard (2.50). Fresh walnuts or dried walnuts have PER value beyond the standard; thus walnuts can be categorized as a source of quality protein.

Keywords: AIN, PER (Protein Efficiency Ratio), protein quality, walnuts (*Canarium vulgare* L.), white rat growth

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INTRODUCTION

Walnuts are indigenous plants of Indonesia that grow in many areas of eastern Indonesia, such as Sulawesi, Maluku, and North Maluku. According to Özdemir and et al (2018), walnuts are tropical plants belonging to the *Burseraceae* family, the *Canarium* genus and have 100 species mostly growing in lowland moist forests in the Melanesian area. Walnuts (*Canarium harveyi*) come from Indonesia, Papua New Guinea, Salomon and Vanuatu (Thomson and Evans 2006). Results of a study by Mailoa et al. (2017) showed that the walnuts (*Canarium vulgare* L) are rich in protein. Fresh walnuts contained 8.2% protein, while the dried ones, roasted in the sand, contained 12.1% protein.

Protein serves as a major structural component of muscle and other tissues in the body. Thus, the growth of animals is closely related to the availability of protein in the feed (Pratama et al. 2015), and growth is one indicator of optimal protein utilization (Siddqui et al. 2014). According to Hossein et al. (2014), Protein Efficiency Ratio (PER) is a measure of the quality of dietary protein, as determined by the ability of these proteins to produce growth in white rats. This study aims to analyze the protein quality of fresh walnuts and dried walnuts to the growth of newly weaned white rats by measuring their growth rate after adding these to their feed.

MATERIALS AND METHODS

Materials

The materials used were walnuts (*Canarium vulgare* Leenh) harvested from Lilibooy Land, Leihitu Barat District, Central Maluku Regency, Maluku Province, and then processed into either fresh walnuts or roasted in the sand to produce dried walnuts. To produce the latter, the ratio of walnuts and sand was 1 : 2, roasted at a temperature of 80 ± 2 ˚C. Other materials used were AIN 93 M feed, casein 9%, fresh walnuts 1.8 g per 20 g of feed, dried roasted walnuts 1.8 g per 20 g of feed and drinking water.

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Table 1. Feed Composition based on AIN 93 M Plus Treatment (every 1000 g of feed)

		AIN Feed + Casein (Control) (g)	AIN Feed + Fresh Walnuts (g)	AIN Feed + Dried Walnuts (g)
Corn Starch	:	620.7	620.7	620.7
Casein	:	140	140	140
Sucrose	:	100	100	100
Sunflower Oil	:	40	40	40
Cellulose (CMC)	:	50	50	50
Mineral Mix	:	35	35	35
Vitamin Mix	:	10	10	10
Casein (9%)	:	90	-	-
Fresh Walnuts	:	-	90	-
Dried Walnuts	:	-	-	90
		1085.7	1085.7	1085.7
Protein Content (%)		11.21	15.11	19.03

The animals test used were male Wistar strain white rats (*Rattus novergicus*) which just weaned for 21 days and adapted for 4 days, with relatively similar average weights of 50.22 to 53.97 g, and where the weight variation of the white rats was not more than 10 g. The animals test were obtained from the Zoological Laboratory, Faculty of Mathematics and Science, Pattimura University, Ambon, Indonesia.

Animal Treatment

Individual cages were used in this study, sized 40 x 30 x 20 cm, with the base of the cage sterile husk. A total of 12 newly weaned (21 days) male Wistar strain white rats (*Rattus novergicus*) were used in this study. After they were weaned, there was a period of adaptation for 4 days before administered the treatment. The rats were divided into three groups of four individuals, and each group received different feeding treatments. The control group (T0) was given normal AIN feed supplemented with 9% casein, the second group (T1) was given normal AIN feed supplemented with fresh walnuts (1.8 g per 20 g of feed), and the third group (T2) was given normal AIN feed with roasted walnuts (1.8 g per 20 g of feed). **Table 1** provides the complete profile of the feeding regimens.

The weight of the rats was measured every two days and the feed consumption measured daily. The rats were fed as much as 25 g per individual per day at 07:00 to 08:00 local times of eastern Indonesia, with drinking water available ad libitum.

Measurement of Protein Efficiency Ratio (PER)

After measuring the gained weight (g) and the protein consumption (g), PER was calculated using the following formula:

$$\text{PER} = \frac{Gained\ Weight\ (g)}{The\ amount\ of\ consumed\ protein\ (g)}$$

The calculations were performed for each rat and the mean values were calculated for each group.

Statistical Analysis

The study design was a completely randomized design (CRD), with three treatment levels (T0, T1, T2) replicated 4 times. Data were analyzed by a one-way ANOVA using SPSS 16 (Sheridan and Steed 2009), to find out the effect of treatment on the parameters tested. If there was a significant difference ($P < 0.05$), further tests using Tukey's HSD Test was performed to compare treatment differences.

Table 2. Composition of Fresh Walnuts and Roasted Roasted Seeds[1]

Composition	Fresh Walnuts	Dried Roasted Walnuts
Water Content (%)	25.0	2.4
Fat Percentage (%)	43.5	71.3
Protein Percentage (%)	8.2	12.1
Digestibility of Protein (%)	84.5	95.3
Trypsin Inhibitor (U/ml)	3.0	1.2

[1] Mailoa et al. (2017)

Table 3. The Total of Feed Consumption and Protein Consumption by Group of Treatment

Treatment	Total Feed Consumption (g/28 days)	Daily Feed Consumption (g/day)	Total Protein Consumption (g/28 days)	Daily Protein Consumption (g/day)
T0	533.76 ± 26.28	19.06 ± 0.94	52.83 ± 2.95[a]	2.14 ± 0.12[a]
T1	554.80 ± 30.28	19.81 ± 1.08	83.83 ± 4.58[b]	2.99 ± 0.16[b]
T2	503.59 + 20.09	17.98 ± 0.72	95.83 ± 3.82[c]	3.42 ± 0.14[c]

T0 = AIN Feed + Casein; T = AIN Feed + Fresh Walnuts; T2 = AIN Feed + Dried Walnuts

RESULTS AND DISCUSSION

Feed Consumption and the Increase of the Rats' Weight

Table 2 shows the nutritional composition of fresh walnuts and dried walnuts, including water content, protein content and fat content, as well as protein digestibility and trypsin inhibitors. The results shown in **Table 3** show that treatment had a significant effect ($P < 0.05$) on daily feed intake and total feed consumption for 28 days. Further test results with Tukey test showed that the amount of feed consumption between treatment types was not different. The milk protein casein was added to AIN feed in the control treatment, as it is used for growth.

The average daily feed intake of the three treatments was normal (17.98 to 19.81 g) in accordance with the average daily feed intake during the growth period of rats of 15 to 20 g for males and 10 to 15 g for females (National Research Council, 1978). However, if these data were compared with protein consumption data, there was a difference between treatments, where the amount of protein consumption at T2 treatment was higher followed by T1 treatment and T0 treatment (control). This is because quantitatively, the feed on T2 treatment has higher protein content (19.03%) than the T1 treatment (15.11%) and T0 treatment (11.21%), as shown in **Table 1**, so although the average amount of feed consumption was the same between treatments.

The average initial body weight of rats before the experiment was the same, but consuming different feeds had some impact on weight gain. Data in **Table 4** showed that treatment type had a significant effect ($P < 0.05$) on the weight gain during the 28 days of the experiment. Further treatment comparisons with Tukey's

Table 4. The Increase of Rats' Weight by Treatment Group

Treatment	Mean Value of Rats' Weight Before Experiment (g)	Mean Value of Rats' Weight After Experiment (g/28 days)	Mean Value of Gained Weight of Rats (g/28 days)	Mean Value of Daily Gained Weight of Rats (g/day)
T0	53.01 ± 0.89	130.43 ± 7.08[a]	77.43 ± 7.91[a]	5.44 ± 0.41[a]
T2	52.07 ± 1.37	157.17 ± 7.20[b]	105.10 ± 6.29[b]	7.55 ± 0.40[b]
T3	51.85 ± 0.67	174.67 ± 4.84[c]	122.84 ± 4.69[c]	8.77 ± 0.34[c]

T0 = AIN Feed + Casein; T1 = AIN Feed + Fresh Walnuts; T2 = AIN Feed + Dried Walnuts

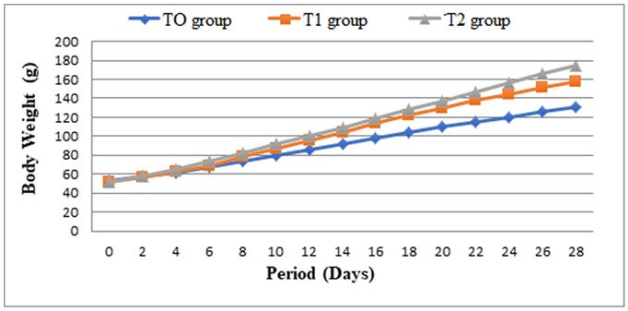

Fig. 1. 28 days of weight growth curve. T0: AIN Feed + Casein; T1: AIN Feed + Fresh Walnuts; and T2: AIN Feed + Dried Walnut

HSD Test indicated that there was a difference between treatments. The increase in body weight of the white rats in the group of rats that consumed AIN feed with dried roasted walnuts was higher followed by rats who consumed AIN feed with fresh walnuts and group of rats in the control treatment. According to Campbell et al. (2007), consuming foods which are rich in protein as the source of amino acids is beneficial to increase muscle mass and maintain immune function.

In addition to the high protein content in the T2 treatment, which was the cause of rat weight gain, it was suspected that there were other factors that could also affect the weight gain. As stated by Mursyid et al. (2014), the proportion of protein, fat, and carbohydrates can also affect growth. The results shown in **Table 2** from previous research demonstrating that the fat content of walnuts was quite high: fresh walnuts: 43.5% and dried walnuts: 71.3% (Işık et al. 2015, Mailoa et al. 2017, Mirzamasoumzadeh and Mollasadeghi 2013). The high content of fat in walnuts was thought to have an impact on the growth of rats because fat is a source of energy that can increase muscle mass and affect weight gain. As stated by Hoffman et al. (2006), if energy intake is inadequate, this will restrict the increase in body mass and muscle mass, even if protein intake is otherwise quite high.

Although the data in **Table 4** indicated that both feeding treatments added with walnuts (T1 and T2) showed higher rat weight results compared with the treatment of non-walnut (T0) control, the weight growth curve shown in **Fig. 1** indicated that treatment control (T0) also had significant weight gain although the overall results were lower.

Table 5. Average PER value by treatment group

Treatment	Protein Efficiency Ratio (PER)	Standard Value
T0	2.51 ± 0.008[a]	
T1	2.53 ± 0.014[a]	2.5
T2	2.58 ± 0.013[b]	

T0 = AIN Feed + Casein; T1 = AIN Feed + Fresh Walnuts; T2 = AIN Feed + Dried Walnuts

According to Smith and Mangkoewidjojo and Smith (1988), the growth rate of rats is 5 g/day. According to this standard, although the weight gain of the rats from the control treatment (T0) were lower than the other treatments, the T0 treatment resulted in weight gain of rats that remained within the normal range of 5.44 ± 0.41 g/day (**Table 4**). The data shown in **Table 4** indicate that the group of rats treated with fresh and dried walnuts (T1 and T2 treatment) showed an average increase in the weight gain that exceeded the normal growth rate, 7.55 ± 0.40 and 8.77 ± 0.34 g/day, respectively. This was also shown in the 28 days of weight growth curve of the rat (**Fig. 1**) in which the weight of the rats treated with T1 and T2 continued to increase and it increased significantly compared to the weight of the rats in the control treatment group. It means that the protein content of both treatments is a quality protein and good enough for the growth of the rats.

Protein Efficiency Ratio (PER)

After measuring the weight gain of the rats and the amount of protein consumed during the 28 days of the experiment, the calculation of protein quality with PER size was performed. The value that obtained from each treatment is compared to the standard value of 2.5. Any value that exceeds the standard is considered an excellent source of protein (Zeren et al. 2016).

The data in **Table 5** showed that the treatment type had a significant effect ($P < 0.05$) on PER. Further treatment comparisons with Tukey's HSD Test showed that T1 vs. T0 (control) did not show a significant difference, whereas the T2 treatment showed significant differences with both. This was due to the higher protein content of the T2 treatment compared with the T1 and T0 treatments (**Table 1**). However, when those compared to the standard PER value (2.5), the PER value of these three treatments is within the fair protein category standard. This indicated that the protein intake contained in the feed material, either from AIN feeds supplemented with casein, fresh walnuts or dried walnuts, can all be well utilized by the experimental white rats for their growth.

One of the indicators of protein quality is the body's ability to digest it (Hoffman and Falvo 2004, Schaafsma 2000). This idea is assumed to be true because the protein digestibility of walnuts is quite high, fresh walnuts have 84.5% protein digestibility while dried roasted walnuts have 96.5% protein digestibility (Mailoa et al. 2017). According to Asrullah et al. (2012), the nutritional value of a protein is determined not only by its protein content but also by whether it can be used by the body.

Protein digestibility defined as the effectiveness of protein absorption by the body. High protein digestibility is also related to low amounts of trypsin inhibitors present in the protein source. According to Gu et al. (2010), the activity of trypsin inhibitors can decrease protein digestibility. Previous research has shown that fresh walnuts or dried walnuts both have low trypsin inhibitor concentrations, 3.0 U/ml and 1.2 U/ml, respectively (Mailoa et al. 2017), thus both have a high protein digestibility (Babu et al. 2009).

CONCLUSIONS

This study demonstrates that fresh walnuts and dried walnuts have PER values above the standard value (2.50), of 2.53 ± 0.014 and 2.58 ± 0.013, respectively; thus, walnuts can be categorized as a source of good quality protein.

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