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Effectiveness of Adas Leaf Infusion (*Foeniculum vulgare L.*) in Post-Best White Rats (*Rattus* Sp.) On The Growth Of Children

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ABSTRACT

Adas leaf (*Foeniculum vulgare* L.) was known by the people around the mountain sides of Merbabu as one type of vegetable to increase breast milk secretion. Increased breast milk secretion has positive effect on pups development in early period of life. The aim of this study is to determine the effect of infusion of adas leaves, the effective dose of infusion against the growth of body weight and body length of pups and the levels of flavonoids, steroids, and stigmasterol on adas leaf. The study was conducted using 12 post-partum rats that have each 5 pups. White rats were divided into 4 groups: control group; the G1 (20 grams/300 ml dose infusion); the G2 (40 grams/300 ml dose infusion); and G3 (60 grams/300 ml dose infusion). Each group consisted of three replications. All of rats mother treated with the adas leaves by infuse feeding (sonde). Adas leaves was infused by 1 ml for 2 times a day on the rats mother (morning and evening). Rats mother were given pellets and also drinking water by *adlibitum*. Both mother and inbred rats were weighed, length of its body weight was measured every 5 days for 15 days. Data were analyzed by *Analysis of Variance* (ANOVA) *two way* and continued by *Duncan Multiple Range Test* (DMRT). Quantitatively measurements of total flavonoids, steroids and stigmasterol of adas leaves analyzed using Thin Layer Chromatography (TLC). The results showed that infusion of adas leaves significant difference in the growth of body weight and body length between control and treatment groups ($p < 0.05$). A dose of 60 grams/300 ml of distilled water can effectively increase the growth of body weight and body length of pups. Levels of total flavonoids, steroids and stigmasterol of adas leaves, respectively, are 0.43 %, 0.029 %, and < 0.011 %.

Introduction

Breast milk is natural baby food which is rich in nutrients and contains higher immunological factors compared to formula milk. Breastfeeding is highly recommended for babies during the first 6 months (exclusive breastfeeding) continued until the child is 2 years old. Breastfeeding in terms of health is very beneficial, because it can reduce infant mortality. In addition, breastfeeding also provides benefits for the mother, including helping uterine involution and spacing pregnancies[1].

Breastfeeding is not only for weight gain, but also provides nutrition to the brain, stimulates the brain automatically to meet the developmental needs of children's skills, intelligence, mental, emotional and social. Not only that, optimal growth and development of children also requires strong nutritional support and stimulation. One of them is by forming and managing children's eating patterns from infancy. Feeding patterns in infants can affect leg length which is a component of body length[2].

Indonesia is a country that is rich in various types of medicinal plants. Some of them are efficacious as lactogogums, such as fennel plants. Lactagogum is a drug that can increase or facilitate milk production. Synthetic lactagogums are not widely known and relatively expensive[1]. The benefits of fennel as an alternative lacticagogue have not received public attention, so that fennel is expected to be used as an alternative lacticagogue in this study.

Fennel (*Foeniculum vulgare* L.) is an annual herbaceous plant originating from Southern Europe and Asia. This plant thrives in areas with an altitude of 1800 meters above sea level and can also grow in the lowlands. Fennel plants are widely grown in Indonesia, India, Europe and Japan because they have many benefits[3]. The part of the fennel plant (*Foeniculum vulgare* L.) used in this study is the leaves. Fennel leaves are obtained from the slopes of the Merbabu mountains. In general, people around the slopes of the Merbabu mountains use fennel leaves as green vegetables. Fennel leaves are also believed by the people around the slopes of the Merbabu mountains to facilitate breast milk for nursing mothers. Sayed et al.[4] explained that fennel plants contain high levels of flavonoids which can affect the endocrine system and hormone functions such as stimulating milk secretion.

Research Malini et al.[5] showed that acetone extract of fennel seeds given for 15 days increased the weight of mammary glands, oviducts, endometrium, myometrium, cervix and vagina. Studies on the potency of fennel leaf infusion for reproductive purposes have not been widely studied. Therefore, this study was conducted to determine the effectiveness of fennel leaf infusion on postnatal white rats on the growth of white rat puppies and compare it with controls.

a) Formulation of the problem
   1. What is the effect of infusion of fennel leaves (*Foeniculum vulgare* L.) on postpartum sows on body weight and body length of white rat (*Rattus* sp.) puppies?
   2. How many doses of fennel leaf infusion (*Foeniculum vulgare* L.) can increase the growth of white rat (*Rattus* sp.) puppies?
   3. What are the levels of flavonoids, steroids and stigmasterol contained in the infusion of fennel leaves (*Foeniculum vulgare* L.)?

b) Research purposes
   1. Knowing the effect of infusion of fennel leaves (*Foeniculum vulgare* L.) on body weight and body length of white rat (*Rattus* sp.) puppies.
   2. Determine the effective dose of fennel leaf infusion (*Foeniculum vulgare* L.) for body weight and body length of white rat (*Rattus* sp.) puppies.
   3. Knowing the levels of flavonoids, steroids and stigmasterol contained in fennel leaf infusion (*Foeniculum vulgare* L.).

c) Benefits of research
Studying the role of fennel leaf infusion (*Foeniculum vulgare* L.) in the reproductive importance of postnatal white rats (*Rattus* sp.) on the growth of tillers.

Method

1. Making Fennel Leaf Infusion (*Foeniculum vulgare* L.)
Making an infusion of fennel leaves uses light green fennel leaves that grow in the middle of the stem. The fennel leaves are air-dried and put in the oven at 40˚C so that the leaves dry with 0% moisture content. After drying, the fennel leaves were blended and sieved using a sieve of size 20 to obtain fine fennel leaf powder. The leaf powder was weighed as much as 20 grams for 20 gram/300 ml infusion, 40 grams for 40 gram/300 ml infusion, and 60 grams for 60 gram/300 ml infusion, accommodated in a beaker glass and mixed with 300 ml of distilled water. After that, it was heated over the bath for 15 minutes at 90˚C while stirring. While it is hot, it is filtered using a flannel cloth. The dregs are added with sufficient hot water and filtered again so that a filtered volume of 100 ml is obtained[6].

2. Treatment
The study was conducted using 12 postnatal white rats with 5 pups each. White rats were divided into 4 groups: group 1 (control), group II (G1) for 20 gram/300 ml fennel leaf infusion, group III (G2) for 40 gram/300 ml fennel leaf infusion, and group IV (G3).) for the dose of fennel leaf infusion of 60 grams/300 ml. Each group consisted of 3 replicates (3 postnatal white rats with 5 pups each). Before being given treatment, the mother white rats were acclimatized for 3 days so they could adapt and not be stressed. The infusion of fennel leaves was given 2 times a day to 1 ml of white rats in the morning and evening. All white rats were treated with an infusion of fennel leaves by force-feeding (sonde). White rats were given rations in the form of pellets and drinking water *adlibitum*. The mother white rats were weighed and their body length was measured every 5 days for 15 days. The body length of white rat puppies (*Rattus* sp.) was measured from the tip of the head to the tip of the tail using a thread to make it more accurate and confirmed by using a vernier caliper.

3. Quantitative analysis of flavonoids, steroids and stigmasterol compounds was carried out using TLC (Thin Layer Chromatography), that is, first, the test solution was prepared by means of 10 mg of ethanol extract and purified extract of fennel leaf powder (*Foeniculum vulgare* L.) respectively dissolved in volumetric flask containing 10 ml of ethanol. Next, a comparison solution of 0.1% fennel leaf powder in ethanol was made, a series of levels was made to obtain an absorption close to the absorption of the test solution. Measurement of the determination of the levels of flavonoids and steroid compounds by preparing 5 µl of each test solution and the series of levels of the reference solution that has been made is spotted on the stationary phase plate using a silica gel 60 F254 plate and eluted with the mobile phase of chloroform : ethanol : glacial acetic acid (94 : 5 : 1v/v). Prior to sampling the fennel leaf powder, the stationary phase must be activated by preheating it in an oven at 110˚C for 15 minutes. This aims to increase the absorption power of the stationary phase. The TLC plate was inserted into the TLC-Scanner tool and the peak area of the standard spot detected by UV light and the fennel leaf powder sample was determined at a wavelength of 425 nm which was controlled via a computer with the winCATS software program. The densitometry data that has been obtained is made from a calibration curve from the ratio of the spotting volume to the peak area and the regression equation is determined. The

regression equation obtained was used to determine the levels of flavonoids, steroids and stigmasterol compounds in the sample[7].

4. Data Analysis

The data obtained in the form of the average value of increasing body weight and body length of puppies in each group were analyzed using a two way *Analysis of Variance* (ANOVA). If an effect is found from this study, proceed to the *Duncan Multiple Range Test* (DMRT) test to see which treatment is effective.

Results and Discussion

Making infusions is a simple way to make herbal preparations from soft materials such as leaves and flowers. Infusion is also called vegetable simplicia which is added to water at 90˚C for 15 minutes[8]. The manufacture of fennel leaf infusion is to determine the effect on the growth of body weight and body length of white rat puppies. The results of research on the growth of white rat puppies, tests for measuring body weight and body length obtained data shown in the form of Table 1 and growth charts as follow Figure 1.

Table 1. Data on the weight of puppies of white rats (*Rattus* sp.) between the control and the doses of fennel leaves (*Foeniculum vulgare* L.) for 15 days

Time	White rat puppies weight (mg)			
	K (Control)	G_1 (20 gram/300 ml)	G_2 (40 gram/300 ml)	G_3 (60 gram/300 ml)
H-5	11444 ± 585	11556 ± 1669	12056 ± 1782	15056 ± 2835
H-10	14689 ± 2136	17111 ± 3698	17278 ± 2616	20000 ± 1756
H-15	23722 ± 3093	23500 ± 4885	23222 ± 3351	28000 ± 3768

The dose given for each treatment showed a different effect on the body weight of white rat puppies for 15 days. Data collection in the form of white rat puppies weight was carried out after 3 days after birth. This is based on research that showed milk production in white rat mothers increases from the 4th to the 10th day of lactation[9,10]. Giving an infusion of fennel leaves (*Foeniculum vulgare* L.) for 15 days because at the age after that, the white rat puppies have left the weaning period. The dose of P2 (40 gram/300 ml) affected the increase in body weight of white rat puppies higher than the control. The increase in body weight of control rats on day 5 was 11444 mg; the 10th day increased by 14689 mg; and on the 15th day it increased to 23722 mg. The control dose showed a relatively constant increase in body weight, while the P2 dose (40 gram/300 ml) showed a significant increase in the body weight of the white rat puppies, namely on the 5th day of 12056 mg; increased by 17278 mg on the 10th day and increased to 23222 mg on the 15th day. The dose of P3 (60 gram/300 ml) was the most effective dose for increasing the body weight of white rat puppies for 15 days, as indicated by the increase in body weight of white rat puppies on day 5 of 15056 mg; increased by 20000 mg on day 10 and increased to 28000 mg on day 15 (Table 1).

Bogart stated the ability of the mother to breastfeed her child, the quantity and quality of feed can affect the weight of the white rat puppies[11]. The body weight of white rat puppies during the weaning period is also influenced by the nutritional content contained in fennel leaves. The nutrients contained in fennel leaves are water, mineral elements, fat, protein, and lactose. Fennel leaves are not only rich in phytoestrogens but also have a high protein content of 22.6%[12]. Protein is needed to increase milk production and the formation of new tissue during breastfeeding and growth[13]. This refers to research by Marwah et al. that supplementation with katuk leaves (*Sauropus androgynus* (L.) Merr) at the beginning of lactation has shown to be effective in increasing the production and composition of Ettawa cross-breed goat milk[14]. The

nutrients contained in katuk leaves and fennel leaves both have an influence on increasing milk production. These nutrients are in the form of protein and other active substances such as beta carotene and steroids. Protein has the effect of stimulating increased milk secretion, while beta-carotene and steroids play a role in stimulating alveolar epithelial proliferation so that new alveoli are formed, thereby increasing the number of alveoli in the mammary glands.

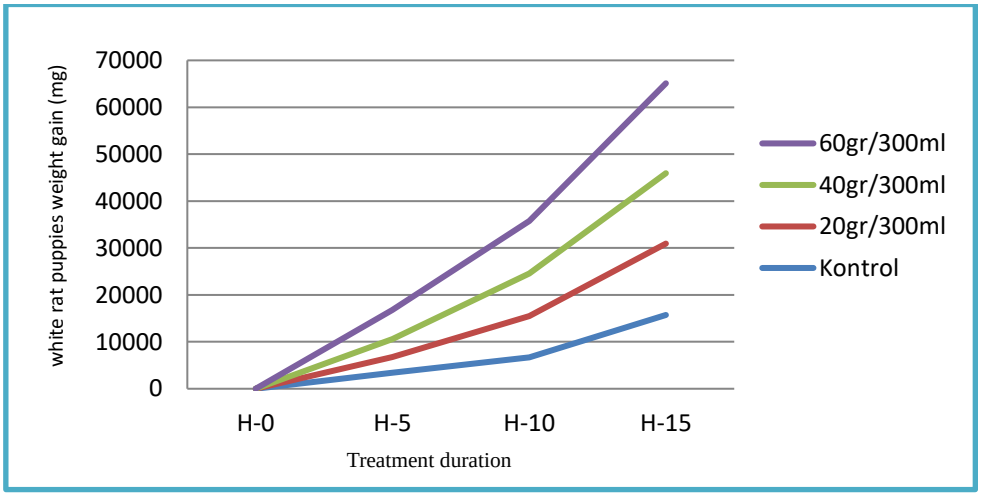

Figure 1. Graph of growth in body weight of white rat puppies for 15 days

Giving an infusion of fennel leaves caused an increase in body weight growth of control white rat puppies from day 0 to day 5 increased by 3444 mg, day 5 to day 10 increased by 6689 mg and day 10 to day 15 increased 15722 mg. The weight growth of white rat puppies dose P1 (20 gram/300 ml) day 0 to day 5 increased 3278 mg while day 5 to day 10 increased 8833 mg and day 10 to day 15 added 15222 mg. The growth in body weight of white rat puppies dosed P2 (40 gram/300 ml) from day 0 to day 5 increased 3834 mg, day 5 to day 10 increased 9056 mg and day 10 to day 3 15 plus 15000 mg. Growth of white rat puppies at dose P3 (60 gram/300 ml) from day 0 to day 5 increased 6223 mg, day 5 to day 10 increased 11167 mg and day 10 to day -15 plus 19167 mg. Graph of body weight growth of control white rat puppies, dose of P1 (20 gram/300 ml), dose of P2 (40 gram/300 ml), and dose of P3 (60 gram/300 ml) showed a sharp increase on day 10 to day 3 -15.

This shows that giving fennel leaf infusion can induce an increase in milk secretion which has an impact on increasing the body weight of white rat puppies due to the phytoestrogen content in fennel leaves. Phytoestrogens have the same compound composition as estradiol, which is the most potent natural form of estrogen[15]. The use of fennel leaves is also very possible to meet the high nutritional needs of white rat mothers, especially during the process of forming milk protein[12]. The phytoestrogen content in fennel leaves (*Foeniculum vulgar*e L.) has the same benefits as the phytoestrogen content in katuk and purwoceng leaves. Research by Sa'roni et al. stated that administration of katuk leaf extract for 15 days could increase milk production in postpartum white rat mothers thereby increasing the body weight of white rat puppies[16]. The increase in milk production in white rat mothers and in body weight of white rat puppies was due to the presence of protein, phosphate, calcium, fat, vitamins, iron, flavonoids, steroids and polyphenols. Research by Setyaningtijas et al. also stated the same results, giving purwoceng ethanol extract orally for 13 days to pregnant white rats stimulated growth and increased body weight of white rat puppies[17]. Purwoceng contains active ingredients that are estrogenic in the form of alkaloids, tannins, flavonoids, triterfenoids, steroids, and glycosides (Fig. 6).

Table 2. Analysis of variance (ANOVA) of body weight growth between the control and treatment groups of fennel leaf infusion (*Foeniculum vulgare* L.) to white rat puppies

Tests of Between-Subjects Effects

Dependent Variable:Berat tubuh (gr)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6050.695[a]	15	403.380	75.554	.000
Intercept	34530.931	1	34530.931	6.468E3	.000
P1	5703.995	3	1901.332	356.125	.000
P2	226.448	3	75.483	14.138	.000
P1 * P2	120.252	9	13.361	2.503	.011
Error	683.384	128	5.339		
Total	41265.010	144			
Corrected Total	6734.079	143			

a. R Squared = .899 (Adjusted R Squared = .887)

The results of Table 2 show that there is a significant difference between the average body weight growth of white rat puppies (*Rattus* sp.) against the doses of fennel leaf infusion (significant = 0.000 <0.05). The duration of treatment (H-5, H-10 and H-15) of fennel leaf infusion showed a significant difference in the average body weight growth of white rat puppies (significant value = 0.000 <0.05). The average growth of white rat puppies (*Rattus* sp.) from the interactions that occurred between the day treatment and the doses of fennel leaf infusion showed a significant difference (significant = 0.011 <0.05).

Furthermore, to find out the combination of the day of administration of fennel leaf infusion and the treatment of doses of fennel leaf infusion (*Foeniculum vulgare* L.) in postpartum white rats (*Rattus* sp.) which has the most effect on the growth of the puppies' body weight, the Duncan Multiple Range Test was performed. (DMRT) 5%. Based on the results of the 5% DMRT test of the average body weight growth of white rat puppies, the notation of 5% DMRT was obtained as shown in Tables 3 and 4.

Table 3. The effect of 15 days of infusion of fennel leaves (*Foeniculum vulgare* L.) on postnatal white rats (*Rattus* sp.) on the weight growth of puppies

Days	Total	Average
H-0	271.08	7.53[a]
H-5	450.72	12.52[b]
H-10	621.36	17.26[c]
H-15	885.96	24.61[d]

Information. Numbers not accompanied by a letter the same in one column means significantly different on DMRT significance level of 0.05

The results of the 5% DMRT notation showed that the length of days (H-0, H-5, H-10, and H-15) given fennel leaf infusion had a different effect on the weight growth of white rat puppies. The longer the day the infusion of fennel leaves was given, the weight growth of the white rat puppies increased. The average weight growth of white rat puppies on day 0 was 7.53 mg lower than H-5, H-10 and H-15 (Table 3).

Table 4. Effect of infusion doses of fennel leaves (*Foeniculum vulgare* L.) on postnatal white rats (*Rattus* sp.) on the growth of puppies

Treatment	Total	Average
K (control)	517.68	14.38[a]
P_1 (20 gram/300 ml)	534.6	14.85[a]
P_2 (40 gram/300 ml)	543.24	15.09[a]
P_3 (60 gram/300 ml)	633.96	17.61[b]

Information. Numbers not accompanied by a letter the same in one column means significantly different on DMRT significance level of 0.05

The results of the 5% DMRT notation showed that the dose of fennel leaf infusion (*Foeniculum vulgare* L.) had an effect on the weight growth of white rat puppies. Treatments K (control), P1 (20 gram/300 ml), and P2 (40 gram/300 ml) had the same effect on the growth of white rat puppies, but had a different effect than P3 (60 gram/300 ml). The higher the dose of fennel leaf infusion, the more the white rat puppies gain weight. This may be due to the content of flavonoids, steroids, stigmasterol and nutrients found in fennel leaves. The content of flavonoids, steroids, and stigmasterol in fennel leaves can increase the milk output of the white rat mother so that the puppies gain weight. The content of flavonoids, steroids, and stigmasterol including estrogenic compounds that can bind to estrogen receptors[18]. Estrogen has two types of receptors, namely estrogen receptors alpha (REα) and beta (REß). α receptors are found in the ovaries, breasts, uterus, and pituitary[19]. Fennel leaves also contain protein which is an important nutrient for living things. In general, white rats need as much as 12% protein a day[20]. The average body weight growth of white rat puppies in the P3 treatment was 17.61 mg higher than the K, P1 and P2 treatments (Table 4).

Table 5. Data on body length of white rat (*Rattus* sp.) puppies between control and treatment with infusion doses of fennel leaves (*Foeniculum vulgare* L.) for 15 days

Time	Body length of white rat puppies (mm)			
	K (control)	G_1 (20 gram/300 ml)	G_2 (40 gram/300 ml)	G_3 (60 gram/300 ml)
H-5	7.7±0.3	7.7 ± 0.4	8.1±0.4	8.4 ± 0.7
H-10	8.5 ±0.3	8.9 ± 0.3	9.0 ± 0.1	9.4 ± 0.9
H-15	10.1±0.6	10.6 ± 0.9	10.0 ± 0.4	10.8 ± 0.7

The dose treatment of fennel leaf infusion was also the most effective in influencing the body length of white rat (*Rattus* sp.) puppies for 15 days, namely P3 (60 gram/300 ml with a total body length of 10.8 mm, while the total body length of white rat (*Rattus* sp.) for 15 days between controls, P1 (20 gram/300 ml) and P2 (40 gram/300 ml) respectively, namely 10.1 mm, 10.6 mm, and 10.0 mm. Table 2 shows between controls, P1 (20 gram/300 ml) and P2 (40 gram/300 ml) from the 5th to the 15th day there was an increase in the body length of white rat puppies, but it was less effective when compared to the dose of P3 (60 gram/300 ml). The most effective dose of P3 (60 gram/300 ml) for increasing the body length growth of white rat puppies, because it is suspected that the phytoestrogens in the form of flavonoids, steroids and stigmasterol found in fennel leaves can increase milk production thereby stimulating the growth of the body length of the puppies white. Fradson stated that Milk production is produced by the mammary glands, m contains protein, fat, carbohydrates, vitamins and minerals which are very important for the growth of children during breastfeeding[11]. This is explained by Anggorodi's research that the growth of children from birth to after weaning is influenced by milk production[11]. The rate of milk production is also affected by the growth of the mammary glands and related hormones. Estrogen and progesterone play a role in the formation of the mammary gland secretion system and prolactin stimulates milk production[21].

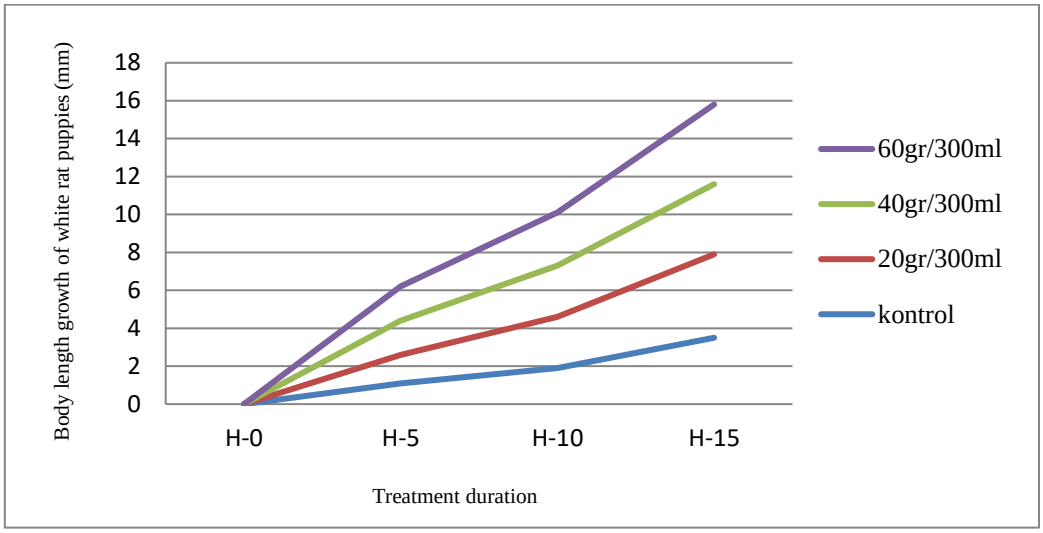

Figure 2. Graph of growth in body length of rat puppies for 15 days

The body length growth of control white rat puppies on day 0 to day 5 increased by 1.1 mm, day 5 to day 10 increased by 1.9 mm and day 10 to day 15 increased by 3, 5mm. The body length growth of white rat pups dosed P1 (20 g/300 ml) on day 0 to day 5 increased by 1.5 mm, day 5 to day 10 increased by 2.7 mm and day 10 until the 15th day increased by 4.4 mm. The dose of P2 infusion (40 gram/300 ml) on day 0 to day 5 increased by 1.8 mm, day 5 to 10 increased by 2.7 mm and day 10 to day 15 up to 3.7mm. The body length growth of white rat puppies at a dose of P3 infusion (60 gram/300 ml) from day 0 to day 5 increased by 1.8 mm, day 5 to day 10 increased by 2.8 mm and day the 10th to the 15th day increased by 4.2 mm. Figure 7 shows that the growth pattern of white rat puppies increased sharply on day 10 to day 15 (end of weaning), after reaching the maximum stagnation for some time it will decrease with age until they do not experience growth when they are adults[22].

Factors supporting the increase in body length growth of white rat puppies are not only influenced by the phytoestrogen content found in fennel leaves, but also the nutritional content of fennel leaf infusion which is properly fulfilled. One of the high nutrients contained in fennel leaves is protein. High protein is needed by the mother during lactation[23]. This is related to research by Edozien and Mahan that giving protein supplements to mother animals such as rats and suckling pigs can increase the amount of milk secretion resulting in an increase in the body length of the puppies[24,25]. The nutritional content of fennel leaves has the same benefits as the nutritional content of red turi leaves which can increase milk secretion in mice. Giving turi leaf extract (*Sesbania grandiflora* L.) with a concentration of 20%, 30%, and 40% can increase milk secretion because of the nutritional content in red turi leaves, especially the protein content which contains lots of amino acids so that it can stimulate the secretion of milk in mice. Apart from protein, turi leaves contain carbohydrates, vitamins, minerals, tannins, saponins, glycosides, and peroxidases[26]. The content of phytoestrogens is able to stimulate the body length growth of white rat puppies because it works by binding to estrogen receptors. There are two types of estrogen receptors in the body, namely α receptors and β receptors. α receptors are abundant in the female reproductive tract. ß receptors are found in the kidneys, bones, intestinal mucosa, endothelial cells, brain and blood vessels[27].

Table 6. Analysis of variance (ANOVA) of body length growth between the control and treatment groups of doses of fennel leaf infusion (*Foeniculum vulgare* L.) on white rat puppies

Tests of Between-Subjects Effects

Dependent Variable:Panjang tubuh (mm)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	311.015[a]	15	20.734	52.905	.000
Intercept	10264.223	1	10264.223	2.619E4	.000
P1	298.388	3	99.463	253.787	.000
P2	7.007	3	2.336	5.960	.001
P1 * P2	5.620	9	.624	1.593	.124
Error	50.165	128	.392		
Total	10625.403	144			
Corrected Total	361.180	143			

a. R Squared = .861 (Adjusted R Squared = .845)

Table 6 shows that there was a significant difference between the average body length growth of white rat puppies in the treatment of fennel leaf infusion doses (significant = 0.000 <0.05) and the average growth in body length of white rat puppies in the long treatment of fennel leaf infusion (H-5, H-10 and H-15) showed a significant difference (significant = 0.000 <0.05), while the interaction that occurred between the day and the dose of fennel leaf infusion treatment (significant = 0.124 > 0.05) on average growth The average body length of white rat puppies showed no significant differences. This may occur because the body's response to the given phytoestrogen content depends on the metabolic and physiological factors of the animal. Phytoestrogens given at the right dose can have a good effect on the hormonal balance in the body, especially during bone growth[28]. The phytoestrogen content contained in the fennel leaf infusion has shown a positive effect on the body length growth of white rat puppies, but it is not optimal according to the growth pattern which is slow at first, then the next phase is faster until it reaches the maximum point, and finally the growth rate decreases[29].

Furthermore, to find out the combination of the day of administration of fennel leaf infusion and the treatment of doses of fennel leaf infusion (*Foeniculum vulgare* L.) in postpartum white rats (*Rattus* sp.) which affect the growth of the body length of the puppies, the Duncan Multiple Range Test was performed (DMRT) 5%. Based on the results of the 5% DMRT test of the average body length growth of white rat puppies, the notation of 5% DMRT was obtained as shown in Tables 7 and 8.

Table 7. Effect of 15 days of infusion of fennel leaves (*Foeniculum vulgare* L.) on postnatal white rats (*Rattus* sp.) on the growth of body length of puppies

Duration	Total	Average
H-0	232.56	6.46[a]
H-5	285.48	7.93[b]
H-10	322.92	8.97[c]
H-15	374.4	10.40[d]

Information. Numbers not accompanied by a letter the same in one column means significantly different on DMRT significance level of 0.05

The results of the 5% DMRT notation showed that the length of day in the infusion of fennel leaves (*Foeniculum vulgare* L.) had an effect on the body length growth of white rat puppies. The length of days (H-0, H-5, H-10, and H-15) given the infusion of fennel leaves, as a whole, had a different effect on the body length growth of white rat puppies. The longer the day the fennel leaf infusion was given, the body length growth of the white rat puppies increased and vice versa. The average body length growth of white rat puppies on day 0 was 6.46 mm lower than H-5, H-10 and H-15 (Table 7).

Table 8. Effect of infusion doses of fennel leaves (*Foeniculum vulgare* L.) on postpartum white rats (*Rattus* sp.) on the growth of body length of puppies

Treatment	Total	Average
K (control)	297.36	8.26[a]
G_1 (20 gram/300 ml)	300.24	8.34[a]
G_2 (40 gram/300 ml)	300.24	8.34[a]
G_3 (60 gram/300 ml)	317.52	8.82[b]

Information. Numbers not accompanied by a letter the same in one column means significantly different on DMRT significance level of 0.05

The results of the 5% DMRT notation showed that the dose of fennel leaf infusion (*Foeniculum vulgare* L.) had an effect on the body length growth of white rat puppies. Treatments K (control), P1 (20 gram/300 ml), and P2 (40 gram/300 ml) had the same effect on the body length growth of white rat puppies, but had a different effect than treatment P3 (60 gram/300 ml).). This is because the higher the dose of fennel leaf infusion given, the longer the body length of the white rat puppies will increase. The average body length growth of white rat puppies in treatment P3 was 8.82 mm, which was 8.82 mm higher compared to treatments K, P1 and P2 (Table 8).

Infusion of fennel leaves for 15 days has shown a different effect on the body length growth of white rat puppies compared to controls. Bone growth is influenced by environmental, hormonal, and genetic factors. Nutrition is one of the environmental factors that affect bone growth and development since prenatal. Protein is a nutrient that can affect bone growth by inhibiting cellular differentiation and changing the rate of bone matrix synthesis. Cellular activity in bone for growth and regeneration is influenced by the hormone estrogen, while collagen and non-collagen proteins each play a specific role in bone formation[30].

The fennel plants containing phytoestrogens could increase bone calcium levels of white rat puppies[31]. The content of phytoestrogens in fennel plants belongs to the lignan group. Lignans are absorbed as secoisolariciresinol and metairesinol, then converted by the intestinal microflora into active estrogen compounds, namely enterodiol and enterolactone. Phytoestrogens can increase the production of insulin-like growth factor (IGF-1) for bone formation. Insulin-like growth factor is a protein that resembles the endogenous insulin hormone and plays an important role in cell growth and metabolism. The content of phytoestrogens can also increase osteoblast proliferation and increase osteoblast differentiation into osteocytes so that bone formation can occur quickly and bone growth will increase[32]. The phytoestrogen content contained in fennel leaf infusion is very dependent on the dose and duration of administration, it takes a relatively long time to see the maximum effect on test animals[33].

Table 9. Phytochemical content of fennel leaves (*Foeniculum vulgare* L.)

No.	Parameter	Fennel Leaves	Moringa Leaves
1.	Total flavonoid	0.43 %	0.65 %
2.	Steroid	0.029 %	1.15 %
3.	Stigmasterol	< 0.011 %	1.52 %

Phytochemical test results obtained from the UGM Integrated Research and Testing Laboratory stated that fennel leaves (*Foeniculum vulgare* L.) contained 0.43% total flavonoids, 0.029% steroids and <0.011% stigmasterol. The highest phytoestrogen content in fennel leaf samples was flavonoid compounds. The total content of flavonoids, steroids and stigmasterols in fennel leaf samples was not as much as the total content of flavonoids, steroids and stigmasterols in katuk and moringa leaves, but could help increase the secretion of mother's

milk and the growth of white rat puppies. In addition to the content of flavonoids, steroids and stigmasterol, fennel leaves also contain other phytoestrogens such as saponin compounds. Phytoestrogens that have not been analyzed such as saponins, are thought to have the highest amount of content in fennel leaves which play an important role in facilitating milk production so that they can increase body weight and body length of white rat puppies. Saponin levels in the seeds of the fennel plant (*Foeniculum vulgare* L.) are 1.35% and 1.07% in the roots of the fennel plant[34]. The leaves, stems, seeds, fruit and roots of the fennel plant contain phytoestrogen components which have a positive effect on the mammary glands and oviducts[35]. Differences in the content of flavonoids, steroids and stigmasterols in a plant material can also be influenced by the growth factors of the plant. Nutrients can affect the levels of phytoestrogens in plants[36]. The total levels of fennel plant flavonoids found on the slopes of the Merbabu mountains were higher than the total content of fennel plant flavonoids found in China. The total level of flavonoid compounds found on the slopes of the Merbabu mountains was 0.43% while the total level of flavonoid compounds in China was 0.0012%[37]. Communities around the slopes of the Merbabu mountains are expected to be able to utilize fennel leaves as a medicinal plant that can increase milk production (Table 5).

Conclusion

Infusion of 1 ml of fennel leaves (*Foeniculum vulgare* L.) twice a day in the morning and evening for 15 days can significantly increase body weight and body length in Rattus sp. The most effective dose of fennel leaf infusion is 60 grams/300 ml with treatment for 15 days. The total levels of flavonoids, steroids and stigmasterol contained in fennel leaf infusion based on the results of quantitative analysis using the Thin Layer Chromatography (TLC) method were 0.43%, 0.029% and <0.011%. Research that can be carried out further is to find out the effects of infusion of fennel leaves (*Foeniculum vulgare* L.) with certain doses on the liver and kidneys of postpartum white rats. Testing the levels of saponins contained in fennel leaves also needs to be done in future research.

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