PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2016.913.920

Research Article
Physiological Effects of Mung Bean Starch RS-3 on the Obesity Index and Adipose Cell Profile of Sprague-Dawley Rats

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Abstract

Objective: This study was conducted to evaluate the physiological effects of mung bean starch RS-3 on the obesity index and adipose cell profile of Sprague-Dawley rats. **Methodology:** A total of 35 male, 1 month old Sprague-Dawley rats were divided into 5 groups and given Fructose Medium Fat (FMF) feed containing Corn Starch (CS), mung bean starch (MS) or Resistant Starch (RS) for 6 weeks. In addition, the diet AIN 93G was used as the standard (STD) feed. **Results:** The results showed that the rats that were fed the RS diet (FMF-RS3-14 and FMF-RS3-28) had a lower feed intake and weight gain compared to those fed the fructose medium fat-corn starch (FMF-CS) or FMF-mung bean starch (FMF-MS) diets. The intervention of the FMF-CS diet led to an increase in the obesity index, whereas, the RS diet decreased the obesity index. **Conclusion:** The replacement of CS with RS at a dose of 28 g/1000 cal (FMF-RS3-28) for 6 weeks was effective in preventing obesity, with a Lee obesity index of 301.8.

Key words: RS-3, mung beans, obesity index, adipose

Received: July 12, 2016 Accepted: August 16, 2016 Published: September 15, 2016

Citation: Priyanto Triwitono, Yustinus Marsono, Agnes Murdiati and Djagal Wiseso Marseno, 2016. Physiological effects of mung bean starch RS-3 on the obesity index and adipose cell profile of Sprague-Dawley rats. Pak. J. Nutr., 15: 913-920.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Obesity is an epidemic health problem of a similar degree for people in both developed and developing countries. Obesity is a risk factor for cancer and cardiovascular disease, which are two of the main causes of death worldwide[1]. At least 3.4 million adults in the world die each year as a result of being overweight or obese[2].

The high prevalence of obesity, particularly in children and adolescents is alarming. It was reported that in 2013, approximately 42 million children under the age of 5 years were overweight or obese worldwide[2]. Up to 18.8% of the children in Indonesia[3] or approximately 8.6 million[4], face this problem. Without professional intervention, those children would be continuously affected by this problem until they reach adulthood[2]. The obvious consequences are that they may develop degenerative diseases both at a higher rate and at an earlier time, thereby shortening their life expectancy[5].

Obesity is an abnormal condition in which an excessive amount of fat is stored in the adipose tissue[6-8]. An unmatched energy balance may cause this circumstance, where the energy intake is higher than the energy to be dissipated. Unreasonable portions at meals and low physical activity are considered the main factors contributing to a surplus energy intake[9]. Therefore, the treatment of obesity using a nutritional approach can be selected as an alternative solution, where the calorie intake is controlled to be less than the total energy released. Whenever the calorie intake is lower, the body fat reserves will then be utilized, thus decreasing the body weight[10].

Dietary Fiber (DF) has a low calorie content[11] only 2 kcal g^{-1}. Resistant starch type 3 (RS-3) is part of the dietary fiber components and is formed by the hydrothermal treatment of starch and retrogradation after cooling[12]. The intake of dietary fiber is known to significantly prevent obesity, increase the sense of satiety and reduce body weight, body fat and body mass index[13]. One indicator of obesity commonly used in rats is the Lee index, which defines the level of obesity of a rat based on its physical parameters[14].

Mung beans are a type of legume that have unique and interesting properties as a source of RS-3. In general, legumes are acknowledged as a significant source of protein. However, mung beans also contain starch and amylose at significantly high concentrations. The higher amylose content will yield a higher concentration of RS-3, as it was formed from amylose broken down via gelatinization and retrogradation processes. In our previous study, it was found that the walet variety possesses the highest potency as a source of resistant starch. The Indonesian walet variety of mung beans has a yield of 35.33% starch, 55.39% db amylose and 15.58% db RS-3[15]. Thus, this study investigated the physiological effects of mung bean RS-3 on the obesity index and adipose tissue profile of Sprague-Dawley rats.

MATERIALS AND METHODS

Materials: The mung beans (*Vigna radiate* var. walet) were a local variety and obtained from Balitkabi (Balai Penelitian Kacangdan Umbi)-Malang, East Java, Indonesia.

RS preparation: Mung Bean Starch (MBS) was extracted using the Hoover method with a slight modification[15]. The RS-3 was obtained using a combination of 2 cycles of autoclaving-cooling and the citric acid hydrolysis of the MBS[16]. A suspension of 20% mung bean starch was heated at 121°C for 20 min by autoclave and then cooled at 4°C for 24 h. This treatment was repeated before continuing to the hydrolysis; 0.1 M citric acid was added at room temperature for 12 h and then the solution was neutralized with 1 M NaOH (pH 7.0) and stored at 4°C for 24 h. The resulting RS-3 was dried at 60°C for 7 h and then pulverized. The RS-3 powder was kept in an airtight container and mixed with other ingredients to formulate the rat feed.

Rat and feed preparation: Male Sprague-Dawley rats (n = 35, weight = 50-60 g, age = 1 month) were obtained from the LPPT Lab., Gadjah Mada University, Yogyakarta, Indonesia. The rats were individually housed in a controlled room (T = 20-25°C and RH = 50-70%) and were divided into 5 groups: Standard (STD), fructose medium fat-corn starch (FMF-CS), fructose medium fat-mung bean starch (FMF-MS), and fructose medium fat-resistant starch (FMF-RS3-14 and FMF-RS3-28). During a week long period of acclimation, AIN 93G was given as the standard feed. After that, during the 6 weeks of maintenance, the STD group was fed the standard AIN-93G feed, the FMF-CS group was fed FMF containing corn starch, the FMF-MS group was given feed containing mung bean starch, the FMF-RS3-14 group was given feed containing 14 g of RS/1000 cal and the FMF-RS3-28 group was given feed containing 28 g of RS/1000 cal.

The composition of the rat feed is presented in Table 1. The feed compositions were designed to have the same numbers of calories (isocaloric); they contained 25% fat (compared to the standard AIN-93G feed with only 7% fat) and fructose was used as the sugar source. The feeding, drinking and recording of the residual feed intake or feed weighing were performed every day, whereas, the weighing and measurement of the body length were performed once a week. At the end of the period of maintenance (end of week 6), the rats were dissected and the adipose tissue was taken for the analysis of the weight and the size and number of adipose tissue cells. The measurements of the rat's body weight, body length and adipose tissue were performed using a scale, a ruler and both a scale and light microscopy, respectively.

Lee obesity index: The Lee index is an index to determine the level of rat obesity based on the ratio between the Body Weight (BW) and the length of the rat's body (LB) using the equation:

$$\sqrt[3]{\left\{\frac{\text{BW}}{\text{LB}}\right\}}$$

Table 1: Feed composition and total calorie

	Feed formulation[a]				
Feed component	STD	FMF-CS	FMF-MS	FMF-RS3-14	FMF-RS3-28
Protein					
Casein (g)	200	200	200	200	200
Carbohydrate					
Corn sarch (g)	397.5	250	0	0	0
Mung bean starch (g)	0	0	250	0	0
RS-3 (g)	0	0	0	400	800
Sucrose (g)	100	0	0	0	0
Fructose (g)	0	100	100	100	50
Maltodextrin (g)	132	100	100	150	0
Fiber (g)	50	50	50	50	50
Fat					
Vegetable oil (g)	70	0	0	0	0
Lard (g)	0	250	250	250	250
Minor ingredients					
Vitamin mix (g)	10	10	10	10	10
Mineral mix (g)	35	35	35	35	35
L-cystine (g)	3	3	3	3	3
Choline bitartrate (g)	2.5	2.5	2.5	2.5	2.5
Total weight (g)	1000	1000.5	1000.5	1200.5	1400.5
Calorie density (kcal g^{-1})	3.93	4.85	4.85	4.04	3.46
Total calori (kcal)	3948	4850	4850	4850	4850

[a]Formulation; STD: Standard, FMF: Fructose medium fat, CS: Corn starch, MS: Mung bean starch and RS: Resistant starch

The body length was measured from nose to anus (ano-nasal) with a ruler in centimeters (cm). A rat is categorized as obese if it has a Lee obesity index[14] greater than 303.

RESULTS AND DISCUSSION

Rat feed

Feed consumption: The desire to eat is known to be influenced by several factors, such as genetics, gender, age, physiological phase (phase of growth, reproduction and lactation) and psychological conditions (affecting the appetite)[17]. The total feed and the average consumption of feed (each week) were not similar, but the amount of each feed consumed seemed to increase with age during the 6 weeks of maintenance (Table 2). The increases in food consumption were because the rats were still in their growth phase (starting from 1 month of age), so they needed a greater feed intake.

Table 2 shows that the dietary intake of the FMF feed was significantly higher than that of the STD feed. The FMF diet contained more fat (25%) than the STD diet (7%). The FMF diet also used beef lard instead of soybean oil in the STD diet. This fat was considered to give a distinctive and better flavor that triggered the rat's appetite.

In the groups of rats fed FMF-CS and FMF-MS, the total feed consumptions were not significantly different. This was due to the difference in the types of starch used in the feeds; the FMF-CS feed contained corn starch and the FMF-MS feed contained native mung bean starch.

In the groups of rats fed the FMF-RS3-14 and FMF-RS3-28 diets, there were significant differences from the STD, FMF-CS and FMF-MS diets. Importantly, RS can swell and has a high water-holding capacity (WHC). When it is hydrated with water or gastric fluids, RS traps the water, causing swelling and a sensation of fullness in the stomach. In this manner, there is competition between the appetite-inducing effects of beef lard and the satiating effects of the gastric filling of RS.

Table 2: Feed consumption and calorie intake during the 6 weeks of maintenance

	Average consumption (g) in 6 weeks							
Feed treatment[a]	1	2	3	4	5	6	Total feed (g)[b]	Total calorie intake (cal)[b]
STD	58.65	53.95	46.85	45.43	55.52	64.57	325.0[a]	1277.1[b]
FMF-CS	52.55	56.38	55.83	64.55	65.22	67.85	362.4[c]	1757.6[c]
FMF-MS	51.15	60.88	61.37	65.02	65.30	67.13	370.9[c]	1798.6[c]
FMF-RS3-14	54.80	53.98	55.88	58.88	57.65	64.48	345.7[b]	1396.6[b]
FMF-RS3-28	48.80	42.23	43.33	56.83	55.68	63.02	309.9[a]	1072.3[a]

[a]Formulation; STD: Standard, FMF: Fructose medium fat, CS: Corn starch, MS: Mung bean starch and RS: Resistant starch, [b]Means of 6 replicates within each column with different superscripts are significantly ($p<0.05$) different

Table 3: Body weight profile and Feed Efficiency Ratio (FER) during the 6 weeks of maintenance

	Body weight (g) in 6 weeks								
Feed treatment[a]	0	1	2	3	4	5	6	Weight gain (g)[b]	FER (%)[b]
STD	71.05	89.53	97.52	106.33	116.75	133.03	151.73	80.68[a]	24.83[a]
FMF-CS	66.83	83.13	98.73	125.40	152.53	184.88	200.30	133.47[c]	34.25[b]
FMF-MS	63.12	84.48	104.52	136.50	157.42	179.13	182.62	121.50[b]	33.57[b]
FMF-RS3-14	58.97	85.35	99.27	123.62	140.28	162.48	173.80	114.83[b]	33.22[b]
FMF-RS3-28	67.90	79.10	84.60	94.14	108.44	123.56	140.48	72.58[a]	23.99[a]

[a]Formulation; STD: Standard, FMF: Fructose medium fat, CS: Corn starch, MS: Mung bean starch and RS: Resistant starch, [b]Means of 6 replicates within each column with different superscripts are significantly ($p<0.05$) different

It is shown that the RS concentration in the feed affected the feed intake. The decrease of the feed intake in the FMF-RS3-28 diet was higher than that of the FMF-RS3-14 diet. In general, the beef lard increased the feed consumption, whereas, the RS decreased the feed consumption.

Calorie intake: The calorie intake as function of the total feed consumption during the 6 weeks of maintenance is given in Table 2. The calorie intake is influenced by the weight and the calorie density (calories per gram) of the feed. The calorie (energy) density is determined by the composition of the feed, mainly carbohydrates (i.e., sugars, dextrin and starch), fats and proteins, which provide physiological energy of 4, 9 and 4 cal g^{-1}, respectively. The RS provides fewer calories, at approximately[11] 2 cal g^{-1}. As a result, the diets containing RS have caloric values lower than the diets without RS. The calorie contents of the FMF RS-14 and FMF RS-28 diets were shown to be much lower than that of the FMF-CS diet.

Calorie intake is required for various activities, particularly basal metabolism, physical activity and growth[17]. In this case, the rats used were still in the growth period (1 month of age). Basal metabolism requires the most calories, up to 60-70% of the total energy, followed by energy for physical activity at approximately[9] 25-35%. The different caloric intakes during the maintenance clearly affected the body weight, body length, obesity index and adipose tissue of the rats.

Feed Efficiency Ratio (FER): The Feed Efficiency Ratio (FER) is a ratio that indicates the efficiency of the feed consumed leading to weight gain during the rat maintenance period, the FER is expressed is as a percent. The FER for the 6 weeks of maintenance is shown in Table 3. The diets that caused higher weight gains were the FMF-CS, FMF-MS and FMF RS3-14 feeds. However, for special purposes such as for weight loss patients who are overweight or obese, the most efficient diets were the FMF-RS3-28 and STD feed formulations. Statistically, both of those had the same physiological effects on the inhibition of weight gain. It can be interpreted that although a consumed diet contains a medium amount of fat (which can be assumed to be delicious, yummy and savory food), such as the FMF-RS3-28 diet, the effect of increasing the body weight can nevertheless be hindered to a level comparable to a low-fat diet by the consumption of fiber (STD diet). In other words, a low-fat diet can promote a normal body weight in the same manner as a moderate-fat diet balanced with high fiber consumption.

Obesity index: The anthropometric parameters to define obesity in animals are limited. However, there are several approaches to determine the index of obesity in rats, e.g., by measuring the abdominal circumference, body length (nose to anus) and body weight. The obesity indicator is then expressed by BMI (body mass index) in g cm^{-2} and the Lee index[14]. A rat is categorized as obese[14,18] if it has a BMI>0.68 g cm^{-2} or a Lee index >303.

Body weight profile: Table 3 shows the weight gains of the rats in the FMF-CS, FMF-MS and FMF-RS3-14 groups were doubled, whereas, the weight gains of the rats in the STD and FMF-RS3-28 groups were significantly lower. The weight gains in the groups of rats fed FMF-CS and FMF-MS were highly significant. The weight gain was positively correlated to both the total feed consumption and the calorie intake.

The rats fed the FMF-RS3-14 and FMF-RS3-28 diets were found to be significantly different in terms of the body weight. The reason for this was that they have different total feed intakes with different caloric values and different contents of digestible and indigestible carbohydrates. The ratio of indigestible carbohydrates in the FMF-RS3-28 feed was much higher than that of the FMF-RS3-14 feed, so the caloric value and the weight gain response were significantly low. The weight gain responses of the rats after the 6 weeks of maintenance are shown in Fig. 1.

Body length profile: In addition to the effect of the weight gain, the feed intake also affected the body length of the rats, as shown in Table 4. In general, the increase in the body length each week was correlated to the increases in the age and feed intake.

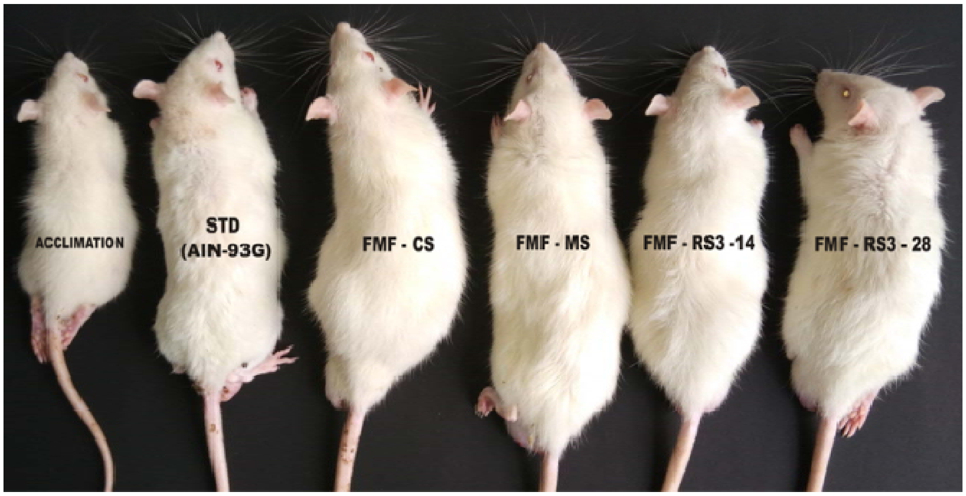

Fig. 1: Visual appearance of the rats after the 6 weeks of maintenance

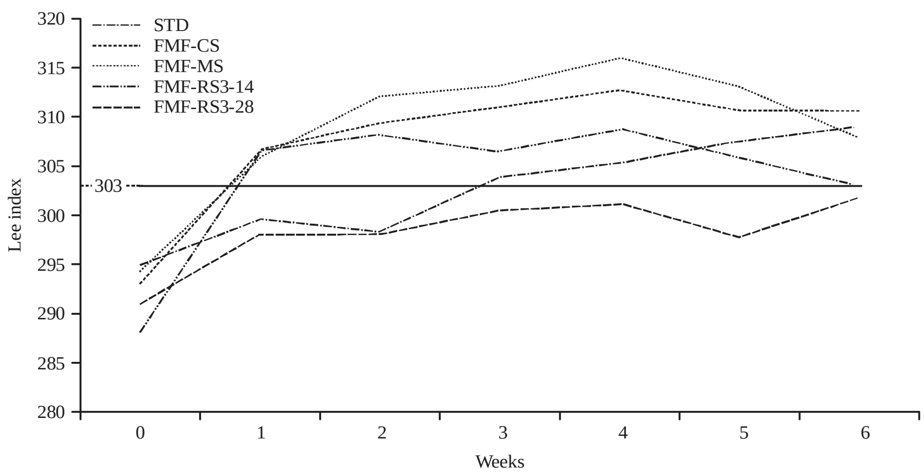

Fig. 2: Lee obesity index during the 6 weeks of maintenance

Table 4: Body length profile during the 6 week of maintenance

	Body length (cm) in 6 weeks						
Feed treatment[a]	0	1	2	3	4	5	6
STD	14.00	14.92	15.42	15.58	16.00	16.58	17.25
FMF-CS	13.83	14.25	14.92	16.08	17.08	18.33	18.83
FMF-MS	13.52	14.33	15.08	16.42	17.08	18.00	18.58
FMF-RS3-14	13.50	14.33	15.00	16.25	16.83	17.83	18.42
FMF-RS3-28	14.00	14.40	14.70	15.10	15.80	16.70	16.90

[a]Formulation; STD: Standard, FMF: Fructose medium fat, CS: Corn starch, MS: Mung bean starch and RS: Resistant starch

Lee obesity index: The body length together with the body weight is an important indicator to measure the level of rat obesity. The Lee obesity index during the 6 weeks of maintenance is presented in Fig. 2. The Lee obesity index showed that the groups of rats fed FMF-CS, FMF-MS and FMF-RS3-14 reached obesity in the first week, followed by the group of rats fed AIN 93 G in the third week. Meanwhile, the group of rats fed FMF-RS3-28 did not reach obesity until the 6th week (Lee index<303).

Because the rats fed the FMF-RS3-14 diet became obese during the first week of maintenance, the role of a normal dose of RS-3 (14 g/1000 cal) was overcome by the effect of the

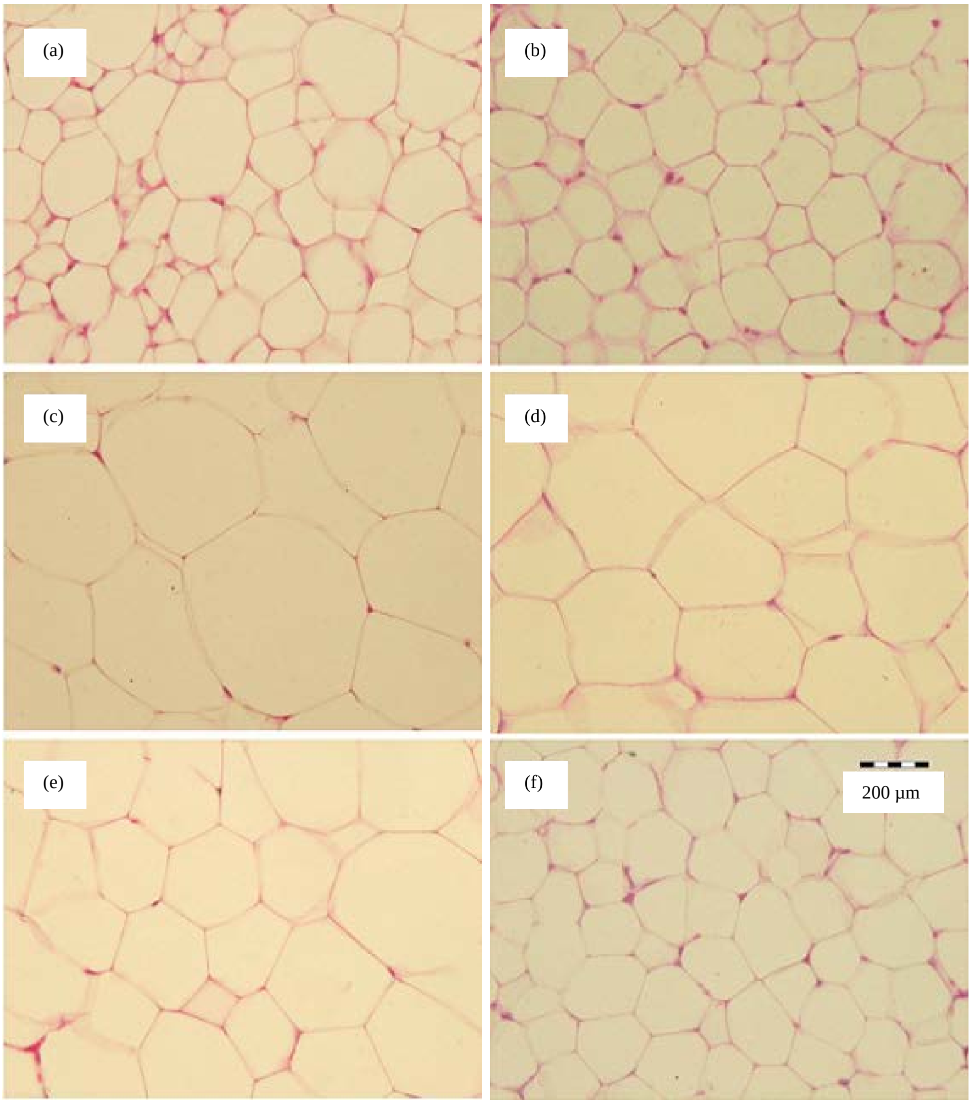

Fig. 3(a-f): Density of adipose cell during the 6 weeks of maintenance, (a) Acclimation, (b) STD, (c) FMF-CS, (d) FMF-MS, (e) FMF-RS3-14 and (f) FMF-RS3-28

Table 5: Weight and cell density of adipose tissue during the 6 weeks of maintenance

Feed treatment[a]	Weight (g)[b]	Increment (%)	Cell density (cell area^{-1})
Week 0			
Acclimation	0.38[a]	0	93
Week 6			
STD	0.77[a]	102.6	68
FMF-CS	2.97[d]	681.6	20
FMF-MS	2.52[c]	563.2	26
FMF-RS3-14	1.68[b]	342.1	31
FMF-RS3-28	0.76[a]	100.0	63

[a]Formulation; STD: Standard, FMF: Fructose medium fat, CS: Corn starch, MS: Mung bean starch and RS: Resistant starch, [b]Means of 6 replicates within the column with different superscripts are significantly (p<0.05) different

FMF diet. Even so, the FMF-RS3-14 diet has been proven to maintain a steady value of the Lee index comparable to that of the STD diet (AIN 93G). This fact can be interpreted that by eating a diet of FMF-RS3-14, the rat can still enjoy delicious fatty food, but its weight is maintained as if it were eating a controlled STD diet. Moreover, the FMF-RS3-28 diet has been proven to be able to withstand the detriments of the FMF diet, so the rats never reached the level of obesity. This finding is interesting for further study and it can be useful for the treatment of obesity, particularly in children and adolescents.

Adipose tissue profile: During the 6 weeks of maintenance, in addition to an increase in the body weight and body length that can be visually observed from the outside, the effect of feeding also can be observed in certain organs such as adipose tissue after dissection (Table 5, Fig. 3).

In general, the accumulation of fat during the 6 weeks of maintenance varied from 100-681.6%. The weight of adipose tissue in the group of STD rats was significantly different than that of the group of FMF-CS rats. This proves that the FMF in the medium fat diet caused the storage of fat in the adipose tissue to a degree of approximately 285.7% or almost tripling the initial weight of the adipose tissue. The accumulation of fat caused the cell size to enlarge, so the cell density decreased from 68-20-26 cells area^{-1} (Table 5). The smaller cell density indicates the greater size of the adipose cells (Fig. 3).

The weight of the adipose tissue in the group of FMF-CS rats was also significantly different compared to that of the group of FMF-MS rats. The two diets can be considered to be similar, but the difference was that the FMF-CS diet used corn starch, whereas, the FMF-MS diet used native mung bean starch. Even so, both corn starch and mung bean starch contribute 4 kcal g^{-1}. Therefore, these results indicate that the difference in the adipose tissue weight was mainly caused by the contribution of calories from fat rather than from starch or carbohydrates. Investigating the cell density, the two groups of adipose tissue were significantly different. The adipose cells of the FMF-CS group had densities lower than those of the FMF-MS group (Table 5, Fig. 3). This means that the size of the rat adipose cells of the FMF-CS group was larger than those of the FMF-MS group. Thus, the adipose tissue of the FMF-CS group was heavier.

Adipose tissue is a fat storage organ. Fat is stored after glucose, in the form of glycogen, in the muscle and liver is completely kept in the organ. There was a correlation between the adipose tissue weight and the body weight of rats. The magnitude of the correlation coefficient was 0.861 and the determination coefficient R2 was 0.7413. This means that 74.13% by weight of the adipose tissue affected the body weight.

When examined, the weights of the adipose tissue of rats fed with the STD diet were not significantly different than those of rats fed with the FMF-RS3-28 diet. This means that neither diet caused a significant accumulation of fat. This fact is in agreement with the cell density data of the rats fed the STD diet (68 cells area^{-1}) and the FMF-RS3-28 diet (63 cells area^{-1}), which were quite similar (Table 5). Contrarily, the cell density of the rats fed the FMF-RS3-28 diet was significantly different compared to that of the rats fed the FMF-CS diet, as shown in Fig. 3. The amount of fat deposited reached 390.8% (almost four times) of the adipose tissue of the rats fed the FMF-RS3-28 diet. If obesity is assumed to be caused by an accumulation of fat, the FMF-RS3-28 diet can be considered to be able to prevent obesity.

CONCLUSION

During the 6 weeks of maintenance, the administration of the FMF diet led to an increase of the obesity index, as observed from the weight gain, the accumulation of fat in the adipose tissue and the increment of the cell size or decreased cell density. To the contrary, the administration of RS caused a decrease in the obesity index which is visible from the weight loss and the decrease of the adipose tissue cell size or increased cell density. The addition of RS at a dose of 28 g/1000 cal or the FMF-RS3-28 diet, which has the lowest FER, proved to be the most efficient way to prevent obesity, as indicated by achieving Lee indexes less than 303.

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