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Study the Effect of Fat Substitutes on Weight Gain in Rats Consuming High Fat Diets

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Abstract:

In the present study, the effect of fat substitutes on weight gain in rats consuming high fat diets was investigated.Biological evaluation, organs weight, lipid profile, blood glucose and histopathological structure of rat's liver fed on high fat diet, were determined. Thirty adult female albino rats, weighting (115±5g), rats were divided into five groups which fed on high fat diet and 10% fatsubstitutes (palm oil, dextrin, whey protein and cellulose).Results showed that feeding rats on 10% Palm oil showed non-significant changes, while10% cellulose significantly decreased BWG as compared to negative control group.For lipid fractionsand blood glucose, the best result showed in rats fed on basal diet with 10% cellulose.Histopathological examination of liver showed less or more normalized in the groups fed on fat substitutes. Thus, the study demonstrated that fat substitutes possesses the ability of weight reduction and decreased the bad effect high fat diet in rats.

Keywords: Biochemical analysis, cellulose, weight reduction.

Introduction

Excessive weight gain arises from the interactions among environmental factors, genetic predisposition and individual behaviors **(DeehDefo*et al.*, 2017).**

Overweight and obesity have reached epidemic levels and are associated with a cluster of metabolic disorders such as type 2 diabetes, metabolic syndrome, dyslipidemia, hypertension and osteoarthritis. Population consuming diets high in dietary fiber have a lower incidence of these chronic diseases **(Guérin-Deremaux*et al.*, 2013).**

An energy imbalance resulting from a combination of an excessive energy intake and a lack of physical activity is considered to be the fundamental cause of overweight and obesity, although there are a limited number of cases which are due primarily to genetics, medical reasons, and psychiatric illness **(Bleich*et al.*, 2008)**

Increasing evidence shows that changes in protein, carbohydrate and lipid proportions could be a key factor to improve body weight regulation after a weight loss program **(Aller*et al.*, 2011).**

Fat substitutes are substances that look, feel and/or taste like fat. Some have calories, while others are low in calories or are calorie-free. The three main categories of fat substitutes are carbohydrate, protein and fat-based. Common carbohydrate-based fat substitutes include cellulose, protein-based fat substitutes, such as egg white, milk and whey and fat-based fat substitutes, with more fatty acids than triglycerides **(Jacqueline , 2013).**

Carbohydrate-based fat substitutes, such asDextrins: When starch is partially hydrolyzed by the action of acids or enzymes (amylases), it is degraded to maltose, maltotriose, and an oligosaccharide called dextrin **(Antonio and Gustavo, 2017).**

Cellulose, a semi-rigid polysaccharide, that is generally known as dietary fiber is commonly used in protein-polysaccharide complexes. Many physicochemical and physiological functions of cellulose and its derivatives have been reported, including water retention, rheological modification, emulsion stabilization, cholesterol level reduction, and bowel function improvement **(Suchmitt and Turgeon,2011andWu *et al.*, 2009).**

Cellulose is the main building material out of which plants are made, and plants are the primary or first link in what is known as the food chain (which describes the feeding relationships of all living things), cellulose is a very important substance **(Bochek, 2003).**

Protein-based fat substitutes, such aswhey protein: whey protein is a protein complex that contains a number of basic amino acids and is the fastest protein that gets into the blood. A popular supplement, whey, has general health benefits in the number of processes and systems such as bone, muscle, blood, brain, pancreas, immune, cancer, infection, metabolism, wound healing, learning and aging **(Mor and Ipekoglu, 2018).**

Shankar and Bansal, (2013) provided that whey proteins help to control the blood glucose levels and provide additional benefits for weight management.

Fat-based fat substitutes, such as Palm oil: PO is entirely GMO-free and produces up to 10 times more oil per unit area than other oilseed crops. In 2012 PO accounted for 32% of global fats and oils production and it has overtaken soybean oil as the most important vegetable oil in the world **(Mba*et al*., 2015).**

Besides having a balanced fatty acid composition, palm oil is also rich in a number of phytonutrients— carotenoids, tocopherols, tocotrienols, sterols, squalene, coenzyme Q10, phospholipids, and polyphenols. Although these minor components constitute less than 1% of the oil, they nevertheless play an important role in the stability and quality of the oil. In addition, all these phytonutrients have antioxidant properties and some of them exhibit nutritional and health benefits beyond their antioxidant function **(May and Nesaretnam, 2014).**

FAs in the 2% position might have an enhanced absorption and thus some researchers have suggested that the palmitic acid in palm oil may be less hypercholesterolemic and atherogenic than that in animal fat. A more recent study found that palmitic acid in the sn-2 position could decrease postprandial lipemia in humans

(Sanders *et al*., 2011). *Trans* Fat increases LDL cholesterol and decreases HDL cholesterol **(Mozaffarian*etal*., 2006).** Palm oil has been suggested as an alternative for partially hydrogenated fats in the food supply to reduce *trans*fat intakes while maintaining the sensory characteristics of foods **(Pedersen *et al*., 2005).**

Materials and Methods

Palm oil, dextrin, cellulose, whey protein, casein, starch, vitamins and salt mixture were obtained from Gomhoria Co. Dokki, Giza.

Thirty adult female albino rats, weighting (115±5g), were obtained from Institute of Ophthalmology, Medical Analysis Dep., Giza, Egypt.

Rats were housed in wire cages under the normal laboratory condition and were fed on standard diet for a week as an adaptation period. Diet was offered to rats in special food cups to avoid looser conditions of food, water was provided to the rats by glass tubes supported to one side of the cage, food and water provided ad-labium and checked daily.

Experimental design

The experimental was done in the Faculty of Home Economics, Menoufia University, Shebin EL-kom. Rats were housed in wire cages in a room temperature 25^0 Cand kept under normal healthy conditions.Rats were divided into the following groups:

Group 1: Rats fed on high fat diet as control group (6 rats).

Group 2: Rats fed on high fat diet with 10% palm oil as a fat source(6 rats).

Group 3: Rats fed on high fat diet with 10% dextrin as a carbohydrate source (6 rats).

Group 4: Rats fed on high fat diet with 10% cellulose as a carbohydrate source (6 rats).

Group 5:Rats fed on high fat diet with 10% whey protein as a protein source(6 rats).

During the experimental period **(28 days),** the diet consumed was recorded every day and body weight was recorded every week. The body weight gain **(BWG),** feed efficiency ratio

(FER), and relative organ weight were determined according to **(Chapman *et al.*, 1959).** Using the following equations:

BWG = Final weight – Initial weight

$$\textbf{FER} = \frac{\textbf{Gain in body weight (g)}}{\textbf{Food consumed (g)}}$$

$$\textbf{Relative organs weight} = \frac{\textbf{Organs weight} \times \textbf{100}}{\textbf{Final weight}}$$

Blood samples were collected after 12 hours fasting at the end of the experimental using the abdominal aorta in which the rats were scarified under ether anesthetized. Blood samples were received in to clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 rpm to separate the serum. Serum was carefully separated, transferred in to clean cuvet tubes, and stored frozen at 20 C for analysis.

Cholesterol, TG, H.D.L-c and L.D.L-c were determined according to **Allain*et al*., (1974)**, **Fossati and Prencipe (1982)**, **Lopez (1977)** and **Lee and Nieman (1996)** respectively.

Serum LDL-c was colorimetrically determined according to the method described by**Friedwald and Levy (1972).** The concentration of the sample was calculated from the following equation:

LDL-c concentration (mg/dl) = Total cholesterol –($\frac{TG}{5}$+ HDL-c)

Serum VLDL-c was color metrically determined according to the method described by **Friedwald and Levy (1972).** The concentration of the sample was calculated from the following equation:

VLDL-c concentration (mg/dl)–$\frac{\mathrm{TG}}{5}$

The principle use of atherogenic index was calculated as the VLDL + LDL cholesterol / HDL ratio according to the formula of **(Kikuchi et al., 1998).**

Serum glucose was measured using the modified kinetic method according to **Kaplan(1984).**

Small Specimens from heart were collected from all experimental groups, fixed in 10% neutral buffered formalin, dehydrated in ascending concentration of ethanol (70, 80 and 90%) cleared in xylene and embedded in paraffin. Sections of (4-6) µm thickness were prepared and stained with Hematoxylin and Eosin according to **Bancroft *et al*.(1996)**.

Statistical analysis were performed by using computer program (Costate) when a significant main effect was detected, the means were separated with the student new mankeuls test. Differences between treatments of ($p \leq 0.05$) were considered significant **(SAS, 1985).**

Results And Dissection

The effect of feeding 10% fat substitutes on Fl, FER and BWG of rats fed on high fat diet.

Data in table (1) indicated that, the mean value of feed intake in the negative control group was 14.32 g/day for each rat, while the mean value of positive control group that is fed on basal diet containing high fat diet was 12.21g/day.

Data also showed that, the mean values of feed intake of groups fed on basal diet and different tested fat substitutes ranged between 12.67 to 15.36g/day.

The highest values of feed intake of rats that treated with diets containing 10% cellulose.From this table, It could be noted that the differences in values of feed intake between all treated groups were significant, as compared to positive control group except group of palm oil. The obtained data revealed a high variation in feed intake between treatments and this may be due to the acceptability of the added materials. These results are in accordance with those reported by **Mba*et al*.(2015)** while these results were not similar to that recorded by **Sanders *et al*.(2011)** who showed that feeding rapeseed diets either as fatand protein decreased the feed intake by 24 and 32% respectively.Results in this table showed a significant increase in FER of positive control (+) as compared to negative control (-).Feeding rats on basal diet containing 10% whey protein resulted in significant changed in FER as compared to positive diet (Control positive) and non-significant with negative control group. The lowest value was recorded in cellulose group.BWG of the group fed on high fat diet (control +) showed significant increase as compared to negative group (control -). Feeding rats on 10% Palm oil showed non-significant changes with the negative control group, while fat substitutes (cellulose) group was significantly lower in BWG as compared to the other groups and the obtained results were in agreement with those reported by **Suchmitt and Turgeon (2011**).

Table (1): The effect of feeding 10% fat substitutes on Fl, FER and BWG of rats fed on high fat diet.

Parameters / Animal Groups	Mean of feed intake (g/day)	FER Mean±SD	BWG (g/28 days) Mean ±SD
CN	14.32±1.11[b]	0.10±0.01[c]	42.27±21.51[b]
CP	12.21±1.23[c]	0.14±0.02[a]	47.87±7.56[a]
10% palm oil	12.68±0.13[c]	0.12±0.01[b]	41.92±5.71[b]
10%whey protein	13.93±2.01[b]	0.09±0.02[c]	37.74±5.26[c]
10% dextrin	14.14±1.91[b]	0.08±0.03[De]	31.04±8.19[d]
10% cellulose	15.36±2.65[a]	0.06±0.01[e]	26.45±9.15[e]

CN **(Control negative)**
CP **(Control positive).**
***Non-significant differences between the values had the same letter.**Significant at p≤0.05

The effect of feeding 10% fat substitutes on relative liver and heart weight of rats fed on high fat diet.

Table (2) represents the effect of feeding different fat substitutes on liver and heart relative weight. The control (-) liver weight was 3.151±0.184g, there is no significant difference (p≤0.05) between control (-) and group fed on the basal diet with 10% cellulose. Also, there is no significant difference between10 % of palm oil group and positive control group. Whereas, there are significant differences between the other groups, positive control and negative control.

In case of heart weight, it could be noticed that there is no significant difference between group fed on 10% palm oil 10%dextrin with values 0, 352±0,018 and 0, 352 ± 0,025. Also,there is no significant difference between group10% whey protein and10% cellulosewith values 0, 0330 ±0,025 and 0, 0334±0,015.

Table (2): The effect of feeding 10% fat substitutes on relative liver and heart weight of rats fed on high fat diet.

Animal Groups	Parameters	
	Liver Mean ±SD	Heart Mean ±SD
CN	3.15±0.18[d]	0.30±0.03[d]
CP	4.08±0.02[a]	0.37±0.01[a]

10% palm oil	3.89± 0.20[a]	0.35±0.02[b]
10%whey protein	3.60±0.12[b]	0.33±0.02[c]
10% dextrin	3.42±0.12[c]	0.35±0.025[b]
10% cellulose	3.25±0.20[d]	0.33±0.01[c]

***Non-significant differences between the values had the same letter.** Significant at $p \leq 0.05$

The effect of feeding 10% fat substitutes on lipid profile of rats fed on high fat diet.

Table (4) illustrated the effect of fat substitutes on the serum cholesterol and triglycerides level of rats.

Data in this table showed that, total cholesterol and triglycerides levels (mg/dl) were increased significantly (P0≤.05) for control positive compared to (control negative).

Total cholesterol and triglycerides were decreased significantly ($P \leq 0.05$) in group fed on basal diet containing 10% cellulose.

The statistical analysis showed a significant decrease in total cholesterol and triglycerides among all treated groups with different fat substitutes when compared with control positive.

In conclusion, treatments under study reduced serum cholesterol and triglycerides concentration. However, the highest reduction was achieved by feeding obese rats with 10 % cellulose followed by 10% of whey protein.

Our results were in accordance with that of **Wu *et al.* (2009)**who found that fat substitutes, cellulose, decreased the high cholesterol level in the blood of rats fed on high fat diet. They suggested that fat substitutesinitiate the lipid transport in the blood and tissue thus leading to the reduction of the cholesterol level. **Mor and Ipekoglu, (2018)**revealed that whey protein help to decreasecholesterol and triglycerides.

Table (3): The effect of feeding 10% fat substitutes on lipid profile of rats fed on high fat diet.

Animal Groups	Lipid Fraction	
	Total cholesterol Mean ± SD	Triglyceride Mean ± SD
CN	89.78±5.25[e]	39.40±0.96[e]
CP	226.55±12.38[a]	189.70±3.11[a]
10% palm oil	189.78±1.72[b]	103.40±2.04[b]

10%whey protein	125.12±4.71^{d}	74.00±3.82^{c}
10% dextrin	154.31±6.15^{c}	79.00±2.85^{c}
10% cellulose	117.48±9.24^{d}	62.10±4.72^{d}

***Non-significant differences between the values had the same letter.**
Significant at p≤0.05

The effect of feeding 10% fat substitutes on cholesterol fractions of rats fed on high fat diet.

Results in table (4) showed the effects of fat substitutes on cholesterol fractions i.e, high density lipoprotein (HDL-c), low density lipoprotein (LDL-c), very low density lipoprotein (VLDL c) and the ratio between LDL-c/HDL-c of rats. The serum HDL-c decreased significantly (P≤0.05) in (control +) that fed on high fat diet compared with healthy rats (control -) that fed on basal diet only.

On the other hand, the mean values of serum HDL-c of all treated rats with fat substitutes were increased significantly (P<0.05), as compared to control (+). The highest mean value of HDL-c was obtained when rats were fed diet containing 10% cellulose as fat substitute, while the lowest mean values of the same parameter was obtained when rats were fed on diet containing 10% palm oils. The results are in the same line with **Wu *et al.* (2009)** who found that HDL-C was higher after feeding cellulose.

High fat diet increased LDL-C in serum rats as compared to normal rats. However, rats that fed on 10% of fat substitutes, cellulose, showed a significant decreased (P≤0.05) in serum LDL-C levels when compared with the other groups. These results appear to be in agreement with those of **Wu *et al.* (2009)** who found that LDL-c decreased significantly due to feeding on cellulose.

Data in same table showed that, the mean values of VLDL-C of rats that fed on basal diet containing high fatsignificantly increased (P<O.05), compared with (control negative group) fed on basal diet only.

Results revealed that, feeding rats with 10% cellulose and dextrincaused significant reduction (P≤0.05) in serum VLDL-C, when compared with control (+) and the other fat substitutes groups.

It could be concluded from the above mentioned results that, all tested groups had sound effect in lowering VLDL-C levels, compared with control (+). Also, the best results in VLDL-C were observed in group fed on cellulose. Our results were in agreement with those obtained by**Suchmitt and Turgeon (2011**).

Results in the same table showed that serum LDL-c/HDL-c ratio increased significantly i($P \leq 0.05$) in (control +) that fed onhighfat diet compared with healthy rats (control -) that fed on basal diet only.

The mean values of the ratio between LDL-c/HDL-camong treated rats with fat substitutes(10% cellulose and whey protein) were decreased significantly, as($P \leq 0.05$) compared to control (+).

This finding was similar to that recorded by **Suchmitt and Turgeon (2011**).

who showed that, cellulose significantly reduced serum LDL-c by 15% and decrease the HDL/LDL ratio by 27%.

Shankar and Bansal, (2013) indicated that in children and familial obesity, a lipid lowering diet with whey protein has a similar effect on total serum cholesterol and LDL-cholesterol compared classical cholesterol reduction diets . However an additional pronounced effect on lowering of triglycerides and VLDL-C can be observed.

Table (4): The effect of feeding 10% fat substitutes on cholesterol fractions of rats fed on high fat diet.

Lipid fraction Animal groups	HDL-C Mean±SD	LDL-C Mean±SD	VLDL-C Mean ±SD	LDL-c/HDL-c Mean±SD
LDL-c/HDL-c	LDL-c/HDL-c	LDL-c/HDL-c	LDL-c/HDL-c	LDL-c/HDL-c
Control (-)	60.58±3062[a]	21.32±2.35[e]	7.88±0.64[e]	0.35±0.32[c]
Control (+)	28.38±5.33[d]	160.23±3.22[a]	37.94±1.33[a]	5.64±1.25[a]
10% palm oil	48.38±4.36[c]	120.72±5.36[b]	20.68±0.24[b]	2.49±0.18[b]
10%whey protein	52.81±7.63[b]	57.51±3.93[d]	14.8±0.16[c]	1.08±0.29[c]
10% dextrin	52.08±4.16[b]	86.43±6.65[c]	15.8±0.32[c]	1.66±0.38[bc]
10% cellulose	53.46±1.94[b]	51.62±1.99[d]	12.4±0.54[d]	0.96±0.53[c]

***Non-significant differences between the values had the same letter.**
Significant at $p \leq 0.05$

The effect of feeding 10% fat substitutes on blood glucose of rats fed on high fat diet.

Data presented in table (5) show the effect of feeding different fat substitutes on blood glucose of rats.

It could be observed that, the mean value ±SD of glucose of (control+ve) group significantly increased, as compared to normal rats, it was being 140.14±0.02 and 80.05±2.11 mg/dl, respectively. In rats given high fat diet then fed on all treatments, there were a significant decrease in the glucose levels as compared to normal group and a significant decrease as compared to positive group.

There is no significant difference between palm oil group and whey protein group. Finally, rats which were given diet contained 10 % cellulose recorded the highestdecrease in glucose level as compared to all the other treatment groups.

Table (5): The effect of feeding 10% fat substitutes on blood glucose of rats fed on high fat diet.

Animal Groups	Glucose Mean ± SD
Control (-)	80.05±2.11[e]
Control (+)	140.14±0.02[a]
10% palm oil	130.13±0.21[b]
10%whey protein	129.11±0.20[b]
10% dextrin	125.79±0.72[c]
10% cellulose	111.9±0.12[d]

*Non-significant differences between the values had the same letter.

Histopathological examination of heart:

Microscopical examination of heart of rat from group 1 revealed the normal histological structure of cardiac myocytes (photos 1 & 2). On the other hand, examined sections from group 2 showed intermuscularoedema (photo. 3) and vacuolation of the sarcoplasm of cardiac myocytes (photo. 4). Some examined sections from group 3 revealed congestion of myocardial blood vessels (photos 5), whereas, other sections from groups 4 and 5 revealed no histopathological changes (photos. 6 and 7).

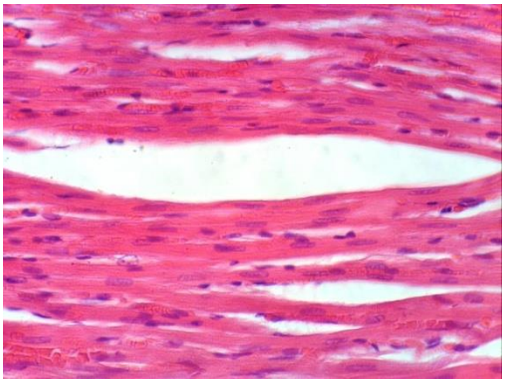


photo. (1): Heart of rat from group 1 showing the normal histological structure of cardiac myocytes (H & E X 400).

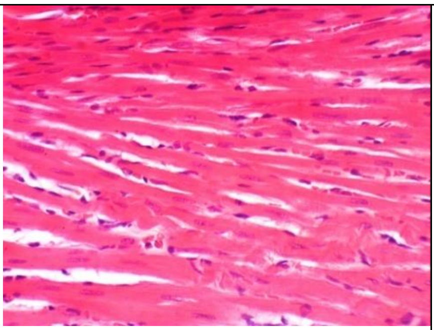


photo (2): Heart of rat from group 1 showing the normal histological structure of cardiac myocytes (H & E X 400).

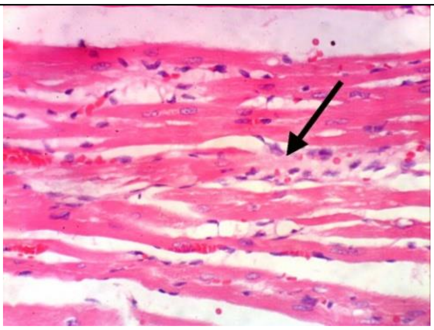
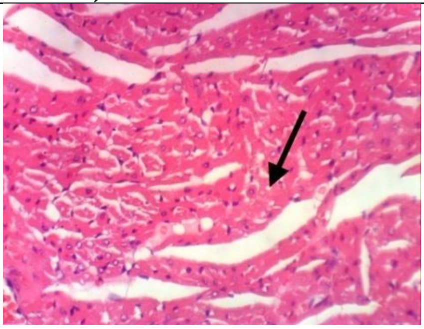


photo. (3): Heart of rat from group 2 showing intermuscularoedema (H & E X 400).



photo(4): Heart of rat from group 2 showing vacuolation of the sarcoplasm of cardiac myocytes (H & E X 400).

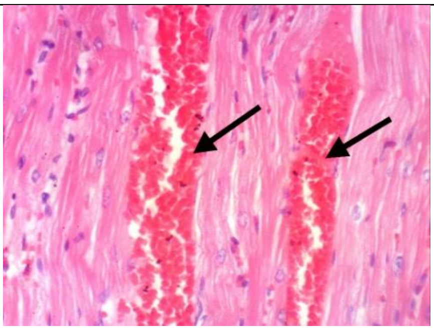


photo (5): Heart of rat from group 3 showing congestion of myocardial blood vessels (H & E X 400).

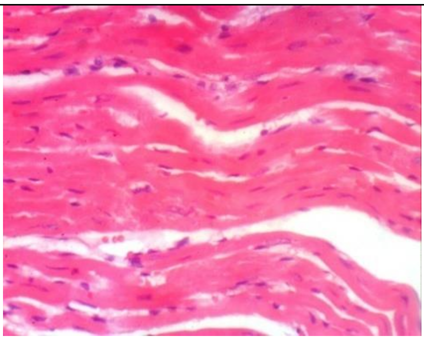


photo (6): Heart of rat from group 4 showing no histopathological changes (H & E X 400).

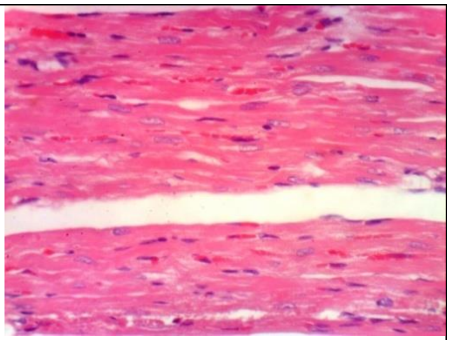


photo (7): Heart of rat from group 5 showing no histopathological changes (H & E X 400).

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دراسة تأثير بدائل الدهون علي الوزن المكتسب للفئران المستهلكة للوجبات عالية الدهن

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الملخص العربي:

تهدف هذه الدراسة الي معرفة تأثير بدائل الدهون علي الوزن المكتسب للفئران المستهلكة للوجبات عالية الدهن.

تم تقدير التحاليل البيوكيميائية ،وزن الأعضاء،دهون الدم،جلوكوز الدم،التركيب الهيستوباثولوجي لكبد الفئران المتغذية للوجبات عالية الدهن.30فأر من اناث الألبينو يزنو5±115 تم تقسيمهم الي5مجموعات حيث تم تغذيتهم علي وجبات عالية الدهن و10%من بديلات الدهن(زيت النخيل،دكسترين،بروتين الشرش والسليلوز).أثبتت النتائج ان الفئران المتغذيةعلي10%من زيت النخيل أظهرت عدم وجود أي تغيرات معنوية بينما 10%سليلوزاظهرت زيادة معنوية في الوزن المكتسب بالمقارنة بالمجموعة الضابطة الموجبة بالنسبة لدهون وجلوكوز الدم اظهرت افضل نتيجة في الوجبة الأساسية مع 10%سليلوز.اظهرت الفحوصات الهيستوباثولوجية للكبد زيادة او نقص في المستوي الطبيعي للمجموعات المتغذية علي بديلات الدهون.اظهرت هذه الدراسة ان بدئل الدهون تمتلك القدرة علي تخفيض الوزن وتقليل التأثير الضار للوجبات عالية الدهون في الفئران.

الكلمات الافتتاحية :التحاليل البيوكيميائية ،السليلوز،تخفيض الوزن.