

## THE EFFECT OF DIETARY HIGH FAT AND LOW PROTEIN ON BODY WEIGHT AND HISTOLOGICAL NATURE OF COLON IN BALB/C MICE.

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### ABSTRACT

The effect of three dietary regimes: - high fat (Hf), low protein (Lp), and High fat low protein (HfLp) was studied on 3 groups of 21 days old Balb/c mice .A fourth group received balanced diet as control (C). histological pictures of large intestine (colon) was examined after 3,6,12 weeks after weaning time (day 0) of experiment .body weight was determined on weekly bases for the four groups.

Significant differences were detected among means of body weights of experimental animals in comparison with control group as following: - Hf>C>HfLp>LP.

Profound histological changes of colon were observed specially in the HfLp group in process of time. Significant differences in numbers of goblet cells and thickness of the adventitial layer of colon was detected, the numbers of goblet cells were reduced in LP group, while their numbers increased in Hf group.

### INTRODUCTION

The incidence of colon cancer is associated with dietary habits (1); epidemiological studies have pointed out that populations consuming diet rich in fat will have a high incidence of colon cancer (2). In experimental models , the effects of dietary variations on carcinogenesis have tentatively explained by modifications in the amount and concentration of total and individual bile acids (3) .Reddy have reported that that population consuming high fat diet show increased levels of bile acids in faeses . and have high incidence of colon cancer (4).On the other hand , protein malnutrition is associated with structural and functional alterations of the intact mucosa of intestine (5).High protein diet stimulates the increasing of some visceral organs such as stomach and large intestine (6).

The purpose of the present study is to indicate the effects of dietary high fat, low protein, or both on body weight and on histological features in Balb/c mice.

### MATERIAL AND METHODS

#### Experimental animals:-

A total of 72 Balb/c mice (male & female) aged 21 days (purchased from Biological and Drug control center/ °wire tops and sow dust bottoms and maintained in constant environmental temperature (25 ± 2°c) with an approximate 12 hours light/dark photo periods.

\*C=Control, Hf=High fat, LP=Low protein, HfLP= High fat Low protein.

\*\*Premixes/kg. diet=vitamin A,6050IU ;vitamin D3,5060IU ;vitamin K ,3.1mg;α-tocopherol,22IU;choline,0.6g;folic acid 2.4mg ;Niacin, 33mg; pantothenic acid ,20mg ;riboflavin ,3.7 ;Thiamin ,11mg ;vitamin B12 , 4.4mg ;Pyridoxine ,1.9 mg; Biotine 0.15; Cobalt,0.44 mg ;Copper,4.0mg ; Iron,132 mg;Manganes ,66mg ;Zinc ,18 mg;Iodin,1.5 mg.

\*\*\* Mj =0.042Kcal/kg.

#### **Histological Technique:-**

The experiment was terminated after 6 and 12 weeks, and animals were killed with chloroform, weighted, and then dissected and portions from colon of control and experimental animal were removed, rinsed thoroughly with normal saline. The tissues were fixed, embedded, and stained with haematoxylin and eosin stains according to (8).

#### **Results and Discussion:-**

**Body Weight:-** Significant differences ( $p < 0.01$ ) in body weight between mice fed on experimental and control diets are clearly detected .Figure (1) shows that body weight of mice fed high fat diet was highly increased as compared with those fed on normal (control) or other experimental diets .This is attributed mainly to the increase of lipid content in different parts of body which is ultimately stored as fat in many parts specially the abdominal region which could be clearly observed during animal dissection. In contrast, a significant decrease in body weight was reported in LP group. Decreased dietary protein results in reduction of cell proliferation and differentiation of some tissues (6). Besides, low protein diet may lead to reduction in pH of stomach and intestine (9) which results in increased peristaltic movement .On the other hand, Ferrell and Kong have reported that high dietary protein results in profound increasing in visceral organ mass, which makes up 10% or less of total body mass (10). While, decreased protein leads to 40-50% reduction in the wet weight of the liver, spleen, intestine, and kidney (5).

**Histological studies:** - Histological studies on sections from colon after 6&12 weeks of nutrient restrictions are shown in figures (2(b, c, d)) as compared with control figure (2(a)).

**Hf group:-** The increasing numbers of goblet cells was the most detectable changes in colon of Hf group (Fig. 3(b)) as compared with control group (Fig. 2(a)) .This increasement may be due to the fact that high dietary fat cause increase in cell division, eventually numbers of epithelial cells will increase ,moreover, fat leads to increased fecal bile acids, which believed to have toxic effect on epithelial cells as it stimulates their proliferation and in turn may stimulate colon cancer(11,12).

Animals divided in to 4 groups according their dietary regimes, each group consisted of 6 cages (3 mice in each cage). Animals fed *ad libitum* diet along the experimental period.

**Diet:-**

Four dietary regimens according to the report of American Institute of Nutrition (7) were prepared as a small pellets by mixing their contents ,kneading with water ,and dried at 60°C for 24 hours .the regimens included:-1)Control group(C), 2)High fat group (Hf), 3) Low protein group (Lp) ,and 4)High fat Low protein(HfLp),table (1).

Table (1): Comparison of experimental diets.

Component	C%*	Hf%	Lp%	HfLp%
Corn	7	28	28	15.75
Wheat bran	64	8.77	15.75	7
Barly bran	8	10	47	48
Milk	1	1	0.75	0.75
Fish( <i>Mugil hishni</i> )	15.5	8.3	1	1
Soy bean	1.5	30.5	3	8
Fat	0	11.6	2	17
NaCl	0.5	0.5	5	0.5
Ca <sub>2</sub> HPO <sub>4</sub>	1.25	1.25	1.25	1.25
Limestone	0.5	0.5	0.5	0.5
Premixes**	0.25	0.2	0.25	0.25
Total protein	20	20	10	10
Total fat	5	20	5	20
Total carbohydrate	59	42	57	43
Total ash	10	8	8	8
Total vitamins	0.1	0.1	0.1	0.1
Total fiber	8	5.6	14	14
Total - digestible energy Mj/100g***	8.412	15.04	8.4	12.33

**Lp group:-** The histological studies of low protein group revealed fusion of the epithelial surface ,and intestinal-walls become thinner ,with few goblet cells, and colon folds seemed to be flattened and have a leaf like appearance (fig.3(C)).These changes may be due to protein deficiency which results in reduction in the brush border region .Also, protein reduction leads to structural changes in tissue formation and this ,in turn , leads to reduction in epithelial cells . Omoik *et al* (1990) have proved through EM study of ileum of protein malnourished animal that micro villi became spare and slender, with only few goblet cells (5).The effect of protein as control factor in proliferation of epithelial cells was not understood, but Jonson (1989) suggested tow factors effecting mucous layer growth: the first one is attributed to non gastric hormones such as thyroxin and somatotropin, the second one is associated with cntry of food to the gastrointestinal tract and neural stimuli(13).

**HfLp group:-** Changes observed in this group were throughout whole structure of tissue ,mucosal folds were fused and shrinked with increased numbers of of goblet cells were clearly detected (fig 3(a)).These changes were resulted from unbalanced state of whole body energy ,as dietary protein reduction leads to reduction in body energy , which is substituted by dietary fat increasment. Crompton (1964) found out that fat increasment must accompany increase in whole body form and structure of different tissues (14).

Further researches are necessary to investigate the effect of dietary restriction on other organs and other tissues.

### تأثير الغذاء مرتفع الدهون منخفض البروتين على وزن الجسم والطبيعة النسيجية للقولون في الفئران المختبرية

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#### الخلاصة

تمت دراسة تأثير الغذاء مرتفع الدهون منخفض البروتين على ثلاث مجاميع من الفئران المختبرية بعمر ٢١ يوم، و عوملت المجموعة الرابعة كمجموعة سيطرة باعطاءها عليقة متوازنة. درست التغيرات النسيجية للأمعاء الغليظة بعد ٣٠، ٢١ اسبوعا ، ووزنت الحيوانات أسبوعيا خلال مراحل التجربة. سجلت اختلافات معنوية في معدلات أوزان الجسم لحيوانات التجربة مقارنة مع مجموعة السيطرة وكالاتي: عالي البروتين السيطرة عالي البروتين منخفض الدهون منخفض البروتين. أظهرت مجموعة الحيوانات المعاملة بالغذاء عالي الدهون منخفض البروتين تغيرات نسيجية في القولون اد كانت هنالك فروقات معنوية في أعداد الخلايا الكاسية وسمك الطبقة البرانية للقولون وقلت أعداد الخلايا الكاسية في مجموعة الغذاء منخفض البروتين بينما ارتفعت أعدادها في مجموعة الغذاء العالي الدهون

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