

# Modification of American Institute of Nutrition Rat Diet in Mammary Cancer Research

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## Abstract

This research was performed to test the effect of date palm fruit (*Phoenix datylifera* L.) of (Al-Barhi) type at the two maturity stages “Rutab” and “Tamr” on the 7,12-dimethylbenz( $\alpha$ )anthracene (DMBA)-induced mammary cancer using the rat model. The experimental diets included soybean group as a control group since soybean is known to be protective against mammary cancer.

The diets, except for the soybean diet, were a modification of the American Institute of Nutrition-1993 (AIN-93) diet that is free of any soybean products. Soybean oil was replaced by a mixture of canola and sunflower oils in amounts that give ratios of  $\omega$ -6: $\omega$ -3 fatty acids and PUFA:SFA similar to those of the AIN-93 diet which are based on soybean oil namely 7.5:1.0 and 4.0:1.0 respectively.

It is concluded that in DMBA-induced mammary cancer experiments, a mixture of canola and sunflower oils in a ratio of 0.6:1.0 (v/v) canola oil:sunflower oil would replace soybean oil.

**Keywords:** American Institute of Nutrition, mammary cancer, rats, diet

## 1. Introduction

Mammary cancer is a neoplastic change in the epithelial cells of the mammary tissue (Hieger, 1961). The use of humans in experimental cancer research is not ethical. This makes the use of animal models for the study of mammary cancer more practical. The use of 7,12-Dimethylbenz( $\alpha$ )anthracene (DMBA) to induce mammary cancer in rodents, is one of the most two commonly used models to study mammary cancer (Guzman et al., 1999).

Several studies examined the antioxidant power of various extracts of the date palm fruit (Qusti et al., 2010; Khanavi et al, 2010). According to the available literature, no study had examined the effect of the whole date palm fruit on mammary cancer *in vivo*. Thus, an experiment was performed to test the effect of date palm fruit (*Phoenix datylifera* L.) of (Al-Barhi) type at the two maturity stages “Rutab” (freeze-dried) and “Tamr” on the 7,12-dimethylbenz( $\alpha$ )anthracene (DMBA)-induced cancer using the rat model.

The American Institute of Nutrition-1993 (AIN-93) recommends the use of soybean oil as the source of fatty acids for growing rats. On the other hand, soybean oil and soy protein isolate were found to be protective against DMBA-induced mammary cancer (Rogers, 1997; Hakkak et al., 2000). This necessitated the replacement of soybean oil with another type of oil which contains similar amounts of fatty acids present in soybean oil.

## 2. Materials and Methods

### 2.1 Materials

Test meals which were fed to the animals were prepared in such a way to be isocaloric and isonitrogenous according to the guidelines of the American Institute of Nutrition (1993) for growing animals (AIN-93G) recommended by Reeves (1997) with modification of the type of oil used.

### 2.2 Area Descriptions

The research was conducted at the Animal Unit at the Department of Nutrition and Food Technology of the

University of Jordan in compliance with the standards of the Deanship of Academic Research.

### 2.3 Methods

The diets which were fed to the experimental animals were prepared according to the AIN-93 guidelines with the following modifications:

1) The soybean oil has been replaced by a mixture of canola and sunflower oils in such way to get ratios of linoleic acid (LA): $\alpha$ -linolenic acid (ALA) and polyunsaturated fatty acids (PUFAs):saturated fatty acids (SFAs) similar to those in soybean oil as recommended by AIN-93.

2) Powdered soy bean seeds, freeze-dried "Rutab", and "Tamr" were added at the level of 30% of the diet mixture weight with completion of macronutrient contents to the recommended weights and correction of the weight of starch, casein, and cellulose (Al-Sayyed et al., 2013) according to the proximate composition of the plant products shown in Table 1.

Table 1. Proximate composition of soybean and date fruits used in the experiment<sup>1</sup>

Component	Composition (g/100 g edible dates)			Composition (g/100g raw soybean seeds)
	Fresh "Rutab"	Freeze-dried "Rutab" <sup>4</sup>	"Tamr" Stage	
Moisture	56.26	7.74	12.22	8.00
Crude protein <sup>2</sup>	1.31	2.76	2.00	33.30
Crude fat	0.18	0.38	0.32	18.00
Crude fiber	2.93	6.18	2.58	9.60
Crude ash	1.09	2.30	1.11	5.20
Carbohydrates <sup>3</sup>	38.21	80.60	81.79	25.90
Energy <sup>5</sup>	159.7	336.86	338.04	398.8

Note. <sup>1</sup> Results are average values of duplicate samples; <sup>2</sup> Protein content was calculated by multiplication of nitrogen content by a factor of 6.25 for date palm fruit and 7.2 for soybean according to Holland et al. (1995);

<sup>3</sup> Carbohydrate content was calculated by difference; <sup>4</sup> Calculated values (except for moisture content); <sup>5</sup> Energy (Kcal.) (calculated by multiplying the carbohydrate and protein contents by 4 and the fat content by 9).

Experiments on mammary cancer using freeze-dried "Rutab" and "Tamr" were based on AIN-93 diet and compared with soybean as control (Al-Sayyed, et al., 2013). Four meals were used in the experiment as follows: AIN-93G, AIN-93G containing soybean seeds (AIN-93G+Soybean), AIN-93G containing freeze dried "Rutab" (AIN-93G+"Rutab"), and AIN-93G containing "Tamr" (AIN-93G+"Tamr"). Since soybean oil was not used in the dates experimental groups, trials were done to obtain the optimum oil characteristics similar to those of soybean oil recommended by AIN-93.

### 3. Results

The LA, ALA, PUFA, SFA contents and their ratios in soybean, canola, and sunflower oils are shown in Table 2. Many trials were done to get similar ratios to soybean oil (Table 3). The results of the study were published elsewhere (Al-Sayyed et al. 2013, 2014a, 2014b).

Table 2. LA, ALA<sup>1</sup>, PUFA, SFA contents and ratios in soybean, canola, and sunflower oils<sup>2</sup>

Type of oil	Content (g)/100 g oil				Ratio of	
	LA <sup>1</sup>	ALA <sup>1</sup>	PUFA	SFA	LA:ALA <sup>1</sup>	PUFA:SFA
Soybean	35.7	4.8	10.1	40.5	7.5:1.0	4.0:1.0
Canola	20.3	9.3	29.6	7.1	2.2:1.0	4.2:1.0
Sunflower	35.3	0.9	36.4	13.0	39.2:1.0	2.8:1.0

Note. <sup>1</sup> LA = Linoleic acid, ALA =  $\alpha$ -Linolenic acid; <sup>2</sup> Reference = USDA (2006).

Table 3. Different combination trials of canola and sunflower oils to get the suitable fatty acid ratios

Ratio of canola oil:sunflower oil	Provided Ratio of	
	LA:ALA <sup>1</sup>	PUFA:SFA
1.8:0.18	2.8:1.0	3.8:1.0
2.5:1.0	3.6:1.0	3.6:1.0
1.3:1.0	4.7:1.0	3.4:1.0
1.0:1.0	5.5:1.0	3.3:1.0
0.7:1.0	6.9:1.0 <sup>2</sup>	3.2:1.0 <sup>2</sup>
0.6:1.0	7.1:1.0 <sup>2</sup>	3.1:1.0 <sup>2</sup>
0.8:1.0	6.4:1.0	3.2:1.0
0.4:1.0	9.4:1.0	3.0:1.0
0.2:1.0	15.8:1.0	2.9:1.0

Note. <sup>1</sup> LA = Linoleic acid, ALA =  $\alpha$ -Linolenic acid; <sup>2</sup> These ratios provide the closest ratios to soybean oil.

#### 4. Discussion

The use of soybean oil in the AIN-93G diets recommended by Reeves (1997) at a level of 70 g/kg diet provides the minimum requirements of LA and ALA (12 and 2 g respectively) for growing rats. Soybean is well-known to be protective food against DMBA-induced mammary cancer (Rogers, 1997; Hakkak et al., 2000). Thus, it was recommended to exclude the use of soybean-derived products (oil and protein isolates) in DMBA-induced mammary cancer experiments (Rogers, 1997; Hakkak et al., 2000). It was decided to use a combination of canola and sunflower oils. It is clear that no vegetable oil would provide the same ratios of LA:ALA and SFA:PUFA. From the different combinations done (Table 3), the mixture of canola oil and sunflower oil of 0.6:1:0 was found to be the optimal mixture that provides the most similar ratios of LA:ALA and PUFA:SFA provided by soybean oil (United States Department of Agriculture [USDA], 2006). It is concluded that exclusion of soybean oil and replacing it by oil mixture from canola and soybean oils would eliminate the bias in using soybean oil in breast cancer experiments.

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