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Effect of Feeding Date Pits on Repartitioning Of Nutrients and Fertility in Rats.

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United Arab Emirates University
Deanship of Graduate Studies

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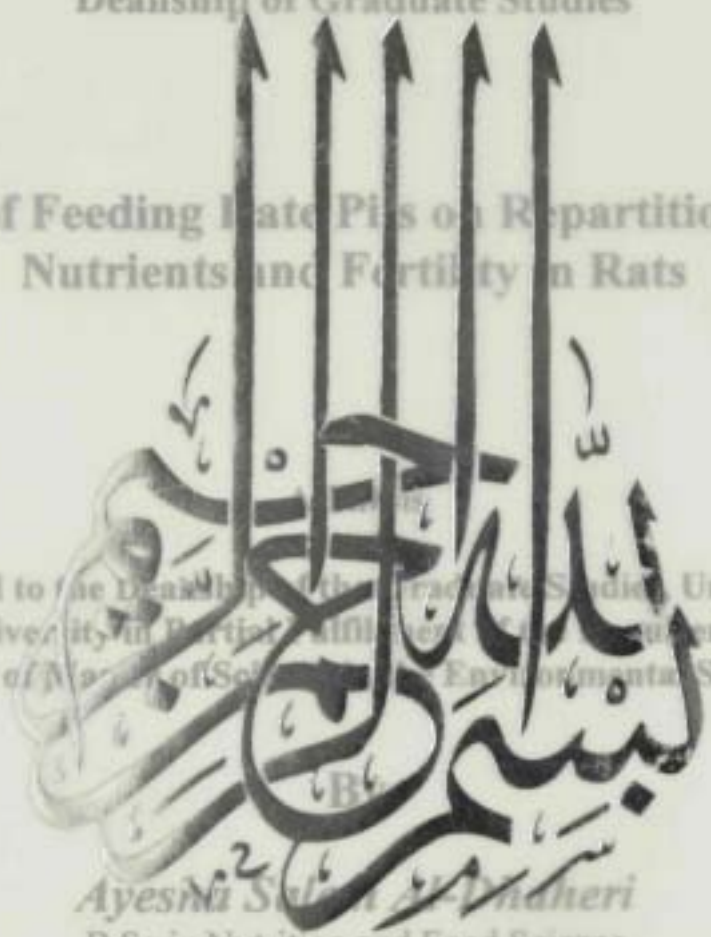

Submitted to the Deanship of the Graduate Studies, United Arab Emirates University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Environmental Sciences

By

Ayesha Sultan Al-Dhaheri

B.Sc in Nutrition and Food Science
Faculty of Agricultural Sciences, U.A.E. University (1997)

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A Thesis

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Title: Effect of Feeding Date Pits on Repartitioning of Nutrients and Fertility in Rats

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Statement by author

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ABSTRACT

ABSTRACT

Five isonitrogenous (23% CP) and isocaloric (2.8 Mcal/kg) diets were prepared and used in this study; two control diets (diet 1, no date pits; diet 2, no date pits + 300 mg/kg vitamin E) and three diets containing date pits (diet 3, 12.5% date pits (DP); diet 4, 12.5% DP + 300 mg/kg vitamin E; and diet 5, 25% DP). Ninety mature Wistar rats (45 females and 45 males) were used in this experiment. All animals were provided with feed and water on an *ad libitum* basis for 29 consecutive days. Water and feed intake were measured daily. Body weights were measured weekly, at the beginning, and the end of each experimental period. Rats were killed by stunning in day 30 and blood samples were collected. Blood serum was analysed for testosterone, oestradiol levels, and for some blood biochemical parameters. Adipose tissue and certain vital organs were excised and weighed. Chemical analyses were done for date pits, experimental diets, and rats carcass to determine crude protein, crude fiber, crude fat, and ash. Results of the approximate chemical analysis of date pits indicated that the nitrogen free extract (NFE) was 71.5 % and only 3 % was starch. When chemical analysis was based on dietary fiber, the NFE was calculated and found to be 26.7% of which 78 % is mannose. Because of the low carbohydrate content in date pits, the total replacement of high energy grain by date pits is not recommended. Feeding

date pits up to 25% to rats enhance the growth of the experimental animals. This may be due to factor(s) other than carbohydrate content in date pits. No effect was observed on water intake. The dietary treatments had no significant effect on feed intake for both male and females rats. The only exception was when vitamin E was added to the control diet in week 1 and 2 in male rats. Also, there were no significant effects on body weight gain for both experimental animals. The only exception was a significant increased in weight gain for male rats in diet 2 during week 1, and for female rats in diet 5 during week 4. The dietary treatments had no significant effect in spleen and adipose tissue weight. Concerning, blood biochemical parameters globulin concentration was within the normal range. Total protein concentration was within the normal range for male rats in diet 4 only, and in diet 3 and 5 for female rats. Diet 1, 2, and 3 for male rats were within the normal range for GPT concentration. The dietary treatments had no effect on testosterone level in male rats. While, oestradiol concentration in the serum of female rats decreased significantly as the percent of date pits increased. The addition of date pits and vitamin E to the diet had the same effect in reducing the oestradiol level in the serum of the female rats, this may be due to estrogenic effect of date pits, which may cause reduction in the fertility of the female rats.

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INTRODUCTION

INTRODUCTION

The date palm *Phoenix dactylifera* is the oldest tree known to be cultivated by man. It is well adapted to the dry and semi-dry regions of the world and mostly found between latitudes 10° to 39° north (FAO, 1999). Date trees are among the oldest cultivated trees and are believed to have been grown in North Africa for at least 8,000 years (Durrani, 2000). Date palms are not grown for their fruit only, but also for several other uses of their parts. Since ancient times, the date palm has been a significant source of food for both human and livestock. Date palm is considered the first fruit crop in the United Arab Emirates (UAE) and occupying 30% of the cultivated land in the UAE (MAF, 2000). The annual production of raw dates reached 757601 tones in the year 2000 (MAF, 2000). Date pits are a by-product of date processing, it is known that the average weight of date pits ranges between 13-15% of the date's weight (Hussein et al., 1998). This makes date pits production around 98488 tones/year; however, very little use is made of these pits. These statistics reflect the importance of date palm trees to the Emirate people who have depended on these trees for centuries.

Date pits are used in the feeding of ruminant animals. Crude protein, crude fat, crude fiber and ash range from 5-7%, 4-10%, 12-27% and 1-2%

respectively. Also, it contains 55-73.7% nitrogen free extract (NFE). Some researchers suggested that the increase in body weight in animals fed date pits was due to it's starch content (55-73.7% NFE) (Al-Azzawi, 1960; Rashid & Alwash, 1976; Al-Asgah, 1987; Yousif et al., 1996; Hussein et al., 1998; and Ali et al., 1999). However, insufficient data are available on the carbohydrates content and the nutritive value of date pits. While other researchers proposed that date pits has an estrogen like substance which act as a phytoestrogens in the body of animals fed date pits (Elgasim et al., 1995; Vandepopuliere et al., 1995; and Ali et al., 1999). The objectives of this Thesis were to: 1) Determine the chemical composition of date pits and its nutritive value in rats; 2) Evaluate the reproductive hormones levels (testosterone and oestradiol) of rats fed date pits.

LITERATURE REVIEW

LITERATURE REVIEW

Date palm

Date palm is one of the most ancient cultivated trees in the world. It is known that the date palm was cultivated as early as 4000 B.C. (FAO, 1999). The date palm (*Phoenix dactylifera L.*) is an economically important fruit tree for many of the populations in North Africa and Arabian countries, and it also has an environmental impact in a desert climate (Ahmed et al. 1995). Throughout the centuries, the date palm has had a long history of religious significance. The Holy Quran mentioned date and date palm in 17 Suras, of the original 114 Suras and 20 verses. In the *Holy Quran* and *Hadith* (the sayings of the Prophet Mohammad), many passages make mention of the importance of the date palm. In the Qur'an, it is called "a blessed tree" when the Prophet built his mosque in Madinah city, the pillars were constructed from the trunks of palm trees and the roof was woven from palm fronds.

A famous reference to the date palm can be found in Surat Maryam regarding Maryam's childbirth: *" And the pains of childbirth drove her to the trunk of a palm tree; she cried (in her anguish) " Ah! Would that I had died before this! Would that I been a thing forgotten and out of sight." But (a voice) cried to her from beneath the (palm-tree): Grieve not! for thy Lord*

hath provided a rivulet beneath thee; and shake towards thyself the trunk of the palm-tree: it will let fall fresh ripe dates upon thee. So eat and drink and cool (thine) eye." (19:23-26)

Another passage of the Quran referring to the date palm: " *And the earth He has put for the creatures. Therein are fruits, date-palms producing sheathed fruit-stalks (enclosing dates).*" (55:10-11)

The botanical name of the date palm, *Phoenix dactylif* presumably derived from a Phoenician name "phoenix", which means date palm, and "dactylifera" derived from a Greek word "daktulos" meaning a finger, illustrating the fruit's form. Belonging to the Angiosperms-Monocotyledones, *Palmaceae* is a family of about 200 genera and 1500 species (Dowson, 1982). Phoenix *(Coryphoideae Phoeniceae)* is one of the genera which contains a dozen species, all native to the tropical or subtropical regions of Africa or Southern Asia, including *Phoenix dactylifera*

In addition to the date palm, five other species of the same genus "Phoenix" bear edible fruit. The five species are 1) *P.atlantica chev* in Occidental Africa and Canary Island; 2) *P.reclinata Jacq.* in Tropical Africa (Senegal and Uganda) and Asia (Yemen); 3) *P. farinifera Roxb.* in India and Ceylon; 4) *P. humilis Royle.* in India and China; and 5) *P. acaulis Roxb* in

India and Bengal. In India fruit from *P. sylvestris Roxb.* is widely used as a source of sugar. *Phoenix dactylifera L.* is distinguished from the above two species by several characteristics such as its dark green leaves, production of offshoots, thick trunk, and its height (if the crown of founds is included, the date palm could reach a height of over 20 m). (FAO, 1999)

Date fruit

Dates fruit consists of 70% soluble carbohydrates (namely glucose and fructose), making it one of the most nourishing natural foods available to man in the dry arid zone. In most varieties, the sugar content of a date fruit is almost entirely of the inverted form. The inverted sugar in dates is easily absorbed by the human body without being subjected to the digestion that other type of sugar undergoes. The water content is between 15 to 30% depending on the variety and the maturity of the fruit. (FAO, 1999)

The flesh of dates contains about 2.5% fiber, 2% protein and less than 2% each of fat and minerals. Date fruits are good source of iron, potassium and calcium. In addition, moderate quantities of chlorine, phosphorous, copper, magnesium, silicon and sulphur are also found in date fruit. Phosphorous content is higher than that found in apricot, pear and grape. It is also high in magnesium (600 mg/1kg of dates). Dates are a good food for those on low sodium diet because they contain very low sodium (1 mg of

sodium per 100g). Dates contain 3 mg/100g of iron, which is almost a third of the recommended dietary allowance (RDA) for an adult male. Furthermore, dates are a good source of vitamins A, thiamin, riboflavin, nicotinic acid (also called niacin), biotin, folic acid, and ascorbic acid. (FAO, 1999)

Dates are eaten mainly as a fresh fruit in many parts of the world. Also, many products are produced from dates such as, date paste, chocolate-coated date, date jams, date butter, date juice and syrup. At the time the United Arab Emirates was founded in 1971 there were 1.5 million date palms in the region (Michael, 1995). Two decades later, the number of date palm trees increased to more than 40 million of which, 16 million were productive (MAF, 2000). The annual production of row dates was 757601 tons in the year 2000 (MAF, 2000). Date palm fruits are considered as one of the most important crops in the United Arab Emirates because of their nutritional and religious importance (El-Behissy et al., 1998). Data published on the nutritional value of dates (Ibrahim & Khalif 1993, and Ahmed et al., 1995) showed clear contribution of dates in the human health when consumed with other food constituents of the daily meals.

Date pits

United Arab Emirates is leading the world in term of date fruit production. This resulted in an increase in the numbers of date factories that produce many date products, leaving a large quantity of seeds as a waste product from date. Date pits are a by-product of processed date. The average weight of date pits ranges between 13-15% of the date's weight (Hussein et al., 1998). This makes date pits production in the UAE around 100.000 tons in the year 2001. Although no part of the tree is wasted, scientists are continually looking for new ways to utilize them. For instance, damaged dates are ground into an additive for animal feed. Ground date pits have been used as charcoal and fertilizer.(Anonymous, 1997[a])

In the last 10 years, some researchers have focused on using date pits in animal feed as a replacement of grain, because it contain 55-70% Nitrogen Free Extract (NFE) which is similar to that of grain. Many investigators assumed that 55-73.7% NFE in the date pits is mainly starch (Al-Azzawi 1960; Rashid & Alwash 1976; Al-Asgah 1987; Yousif et al. 1996; Hussein et al.1998; and Ali et al. 1999). Several studies (Jumah et al. 1973; Kamel et al. 1981; Al-Asgah 1987; Vandepopuliere et al. 1995; and Yousif et al. 1996) studied the effect of date pits on body weight. Rashid & Alawash, (1976) found that the inclusion of date pits in the diet of sheep

may increase body weight gain, improve feed efficiency and enhance meat palatability. Elgasim et al. (1995) found that date flesh and date pits were effective in increasing body weight gain and deposition of back fat of sheep. Hussein et al. (1998) reported that the use of dates and date pits in broiler starter and finisher diets improved the body weight of chicks, total body weight gain and the efficiency of feed utilization. The addition of 14% powdered date pits significantly increased the body weight of rats (Ali et al. 1999). Al-kinani and Alwash (1975) fed date pits to Awassi sheep at level of 0, 25, 50, and 75%, and found that the gain in weight is higher for those groups that fed large proportions of date pits. Shakir et al. (1969 [a]) used concentrate mixtures based on date pits but supplemented with a high protein meal. The concentrate mixtures containing 0, 40, and 80% date pits were given with green alfalfa as forage. The result of that study showed that the growth rates increased as the proportion of date pits in the diet was increased. Al-Azzawi (1960) found a significant improvement upon barley based diet by substitution of 15% date pits in poultry rations. In contrast, Jumah et al. (1973) reported that a broiler diet supplemented with ground date pits at level of 15% resulted in a significant depression in the final body weight and growth. This depression was suggested to be due to a high fiber content of the diets. Al-Asgah (1987) stated that date palm seeds could

replace the commonly used wheat bran-barley mixture up to the level of 75% in fish (*cyprinus* reduce the quality of fish in terms of protein and increase their levels of fats. Kamel et al. (1981) conducted two experiments to investigate the feeding values of date pits and whole dates for broiler chicks. In the first experiment, date pits were included in broiler diets at 5, 10, and 15% replacing wheat bran and corn with and without zinc bacitracin (50 ppm) supplementation. In the second experiment, whole dates were included in four diets at 0, 5, 10, and 47.7%, replacing corn as an energy source. Their results indicated that date pits supported chick growth at all dietary levels tested. Although the whole dates supported growth as efficiently as the control diets, except the incorporation of 47.7% of whole dates resulted in some growth depression and a slight decrease in feed utilization. Vandepopuliere et al. (1995) conducted two broilers growth and two quail breeder studies to evaluate the dietary potential of dates, date meat, and date pits. The test ingredients were integrated in broiler starter diets at levels ranging from 8 to 43% dates, 16 to 43% date meat, and 5 to 27% date pits. The quail breeder diet had ingredient ranges of 10 to 30% dates, 8 to 24% date meat, and 5 to 15% date pits. The result showed that all diets supported broiler weights and feed conversions; however for the quail breeder, there was a decrease in feed consumption at

the 30% date level, which resulted in decreased weight gain. In a study by Yousif et al. (1996) seven isonitrogenous (40% crude protein) and isocaloric (18.6 KJ/g gross energy) diets were prepared to examine the consumption of date and date pits as carbohydrate source for tilapia fry and to estimate the complete substitution of fish meal by poultry byproduct and blood meals. The out come of this study showed that the inclusion of dates and date pits in the experimental diets had no apparent effect on growth performance or feed utilization efficiency of the fish. However, all the experimental diets that were supplemented with date pits showed less lipid content (better performance). Consequently, they concluded that date pits could be incorporated in fish diets as a natural repartitioning agent for obtaining less-fatty fish.

Vitamin E and Fertility

Nutrient (water, energy, amino acid, vitamins, minerals) requirement of animals must be met to satisfy the biological processes associated with maintenance, growth, lactation and reproduction. It is well known that underfeeding during growth period will result in delayed sexual maturity, and usually will result in reduced fertility as compared to that of animals fed on a moderate intake. (Anonymous, 1997[b])

Early vitamin studies identified a substance necessary for animal reproduction that was chemically an alcohol. This substance was named tocopherols, from two Greek words: *tophos*, meaning "childbirth" and *phero*, meaning "to bring" with the *ol* ending for alcohol. Tocopherols soon became known as the antisterility vitamin, but it was soon demonstrated to have this effect only in rats and a few other animals. Tocopherol (vitamin E) is actually the generic name for a group of similar fat-soluble nutrients. Three of these, designated alpha (α), beta (β), and gamma (γ)-tocopherol, display the most biologic activity. Of these three, α-tocopherol is the most significant in human and animal nutrition. (Williams, 2001)

The single vital function of tocopherols relates to its action in many tissues as an antioxidant, which is an agent that prevents cellular structure from being broken down by oxygen. (Williams, 2001)

Many studies have investigated the effect of vitamin E on egg production. Bollengier-Lee et al., (1998) studied the influence of high dietary vitamin E supplementation on egg and plasma characteristics in hens subjected to heat stress. Their data showed that egg production and egg weight were significantly higher ($P<0.05$). Hossain et al., (1998) fed broiler breeders diet containing graded levels of supplementary vitamin E (25, 50, 75 and 100 mg/kg) between 24-54 weeks. Their result showed no significant

difference in percentage of hen daily egg production or number of eggs per hen during the 30 weeks of experimental period. Moreover, Siegel et al., (2001) reported that egg production for broilers fed 300 mg vitamin E was greater than broilers fed 10 mg vitamin E.

A study by Elgasim et al. (1995) showed that the inclusion of date by-products (date pits and flesh) at different levels in the diet of meat animals resulted in increasing weight gain and back fat deposition. Also, they studied the hormonal activity of date and date pits by preparing and adding date pits aqueous extract to a rat's uterus suspended in an organ bath containing De-jalon's solution at 37°C. They concluded that the aqueous extract of date pits acted in a similar fashion to estrogens. Vandepopuliere et al. (1995) fed date pits to breeder coturnix quail to the amount of 5-15% of their total feed and reported that the fertility of those who fed 5% date pits diets was reduced. It is very clear from what had been reported in the literature concerning the effect of date pits on the production and reproductive performance, that the available data are insufficient and inconclusive. In a study by Ali et al. (1999) normal and acid-treated powdered date pits were used in the feed of male rats at two levels (7 and 14%) for 28 consecutive days. They also, treated female rats for 10 days with lyophilized date-pit extract, or with a polar or non-polar fraction. Their data showed an increased in the plasma

concentration of testosterone ($P< 0.05$), while the acid-treated date pits (14%) caused a significant increase in the concentration of luteinizing hormone (LH). Treatment with the date pits did not cause significant changes in any of biochemical plasma parameters measured. Treatment of female rats for 10 days with lyophilized date pits extract, or with a polar or non-polar fraction did not significantly affect the body weight, uterine weight or the opening of the vaginal orifice of the rats. In contrast, treatment with oestradiol (2 mg/Kg^{-1}) for 10 days caused a significant increase in the uterine weight ($P< 0.05$) and the rate of opening of the vaginal orifice.

EXPERIMENTAL METHODS

EXPERIMENTAL METHODS

1 Diet preparation

Five isonitrogenous (23% CP) (Appendix Table C, and Table 4) and isocaloric (2.8 Mcal/kg) (Appendix Table D, and Table 4) diets were prepared; two control diets (control 1, no date pits; control 2, no date pits + 300 mg/kg vitamin E) and three diets containing date pits (diet 1, 12.5% date pits (DP); diet 2, 12.5% (DP) and 300 mg/kg vitamin E; and diet 3, 25% (DP)). Date pits (obtained from Al Ain Date Factory) were ground to 1 mm diameter and used in the experimental diets. Feed ingredients (obtained from Emirates Flour and Animal Feed Factory) were also ground to 1 mm. The diets were formulated and thoroughly hand-mixed with water. The resulting mixture was pelleted to 11 mm in size. Each experimental diets were separately stored in sealed plastic bags until needed. Formulations of the experimental diets are shown in Table 1 (Appendix Table A).

2 Experimental animals

Ninety mature Wistar rats (45 females with an average body weight 118 g $\pm$ 7.5 and 45 males with an average body weight 136 g $\pm$ 9.6) were used in this study. Animals were obtained from the animal facility of Faculty of Medicine and Health Sciences, United Arab Emirates University. Each

3 Experimental procedure

All animals were provided with feed and water on an *ad libitum* basis for 29 consecutive days. Water and feed intake were measured daily. Body weights were measured weekly and at the beginning and end of each experimental period. In day 30 rats were killed by stunning and blood samples were collected from the eye balls in eppendorf tubes. The blood samples were centrifuged at 4000 rpm for 5 minutes. Serum was separated and each sample was divided into two tubes and stored at -18 °C till biochemical and hormonal analysis. One tube from each serum samples was used to determine the concentration of testosterone in male rats and oestradiol hormones in female rats and the other tube was used for biochemical analysis (total protein, albumin, globulin, glutamic pyruvic transaminase, glutamic oxalacetic transaminase, and A/G ratio). Adipose tissue and certain vital organs (liver, kidney, spleen, uterus, and testes) were excised and weighed. The whole animal carcass including internal organs were stored at -18 °C to measure crude protein, crude fat, and ash. Chemical analysis was determined according to AOAC (1990).

%N (DM basis) = V_A × N Hcl × 1.4007/w × Lab DM/100

V_A = Volume, in ml, of standard Hcl required too sample.

N Hcl = Normality of standard acid (Hcl).

1.4007 = Milli equivalent weight of N × 100.

W = Sample weight in grams.

4.3 Crude Fat

Tecator, Soxtec system HT6 was used to determine crude fat. Dried, ground sample (0.5 g) were weighed into thimbles and inserted into the extraction unit. Diethyl ether was used as an extraction solvate. After solvent addition to the extraction cups, the material was extracted into the solvent in a two stage process followed by a solvate recovery cycle. The resulting residue was weighted and referred as ether extract or crude fat.

4.4 Crude Fiber

Crude fiber was determined by using ANKOM 200/220 fiber analyzer. Dried ground samples (0.5 g) were weighed into pre-weighed filter bags. Bags (total of 24 bags) were placed in the bag suspender trays, 1900 – 2000 ml of 1.25% H_2SO_4 solution were added. Agitation and heat were turned on and timer was set for 45 min. After 45 min. heat and agitation were off, the solution was exhausted, and the bags were rinsed with hot water, three times. 1900 – 2000 ml of 1.25% NaOH solution was added.

Agitation and heat were turned on and timer was set for 45 min. After 45 min. heat and agitation were off, the solution was exhausted, and the bags were rinsed with hot water three times. Bags were removed out and placed in a 250 ml beaker, and soaked 2-3 minutes in the acetone. The bags were removed, and gently pressed out for excess acetone. Bags were placed in the oven (150°C) for 2-4 hours. The bags were weighed after and placed in the furnace for 2 hours at 550°C. Weight was recorded, the crude fiber was calculated according to the following equation :

$$CF_{OM}\ (DM\ basis)\ \frac{(w_4 - (w_1 \times C_2)) \times 100}{w_2 \times DM}$$

Where :

w_1 = Bags tare weight

w_2 = Sample weight

w_3 = Weight after extraction process

w_4 = weight of Organic Matter (OM)(Loss of weight on ignition of bag fiber residue)

C_2 = Ash corrected blank bag(Loss of weight on ignition of bag/original blank bag)

4.5 Ash

A dried, ground sample (2.0 g) was weighed in pre-weighed crucible and ignited in a furnace at 600°C for 2 hours. The crucible was removed and transferred to desiccator to cool. The final weight was recorded, and the % of ash was calculated.

5 Serum Biochemical analysis

The blood samples were centrifuged at 4000 rpm for 5 minutes to extract the serum. Serum samples (0.5 ml) were used to determine the following blood parameters:

5.1 Total Protein

Cupric ion (Cu++) reacts with the peptide linkages $(-C(=O)-NH-CHR-C(=O)-NH-)$ of protein in a basic solution. The blue copper (II) protein complex thus formed is proportional to the total protein concentration in the sample and is measured using a bichromatic (540,700 nm) endpoint technique. This test performed on the Dimension ® clinical chemistry system. (Kingsley, 1942)

$$Cu^{++} + \text{Protein} \xrightarrow{OH^-} \underset{(absorbsat\ 540\ nm)}{complex}$$

5.2 Albumin

In the presence of a solubilizing agent, BCP binds to albumin at pH 4.9. The amount of albumin – BCP complex is directly proportional to the albumin concentration. The complex absorbs at 600 nm and is measured using a polychromatic (600, 540, 700 nm) endpoint technique. This test performed on the Dimension ® clinical chemistry system. (Carter, 1970)

5.3 Globulin

Calculated from the subtraction of Albumin from total protein. Globulin =Total protein- Albumin.

5.4 Glutamic Pyruvic Transaminase (GPT = ALT)

Alanine aminotransferase (ALT = GPT) catalyzes the transamination of L-alanine to ∝- ketoglutarate (∝--KG), forming L-glutamate and pyruvate. The pyruvate formed is reduced to lactate by lactate dehydrogenase (LDH) with simultaneous oxidation of reduced nicotinamide-adenine dinucleotide (NADH). The change in absorbance is directly proportional to the ALT activity and is measured using a bichromatic (340, 700 nm) rate technique. This test performed on the Dimension ® clinical chemistry system. (Bergmeyer, 1978)

$$L-alanine+\alpha-KG \xrightarrow[P5P,Tris,pH7.4]{ALT} L-glutamate+pyruvate$$

$$Pyruvate+NAD(H+) \xrightarrow{LDH} Lactate+NAD+$$

5.5 Glutamic Oxalacetic Transaminase (GOT = AST)

Aspartate aminotransferase (AST = GOT) catalyzes the transamination from L-aspartate to ∝- ketoglutarate, forming L-glutamate and oxalacetate. The oxalacetate formed is reduced to malate by malate dehydrogenase (MDH) with simultaneous oxidation of reduced nicontinamide adenine dinucleotide (NADH). The change in absorbance with time due to the conversion of NADH to NAD is directly proportional to the AST activity and is measured using a bichromatic (340, 700 nm) rate technique. This test performed on the Dimension ® clinical chemistry system. (Saris, 1978)

$$L-aspartate+\alpha-Ketoglutarate \xrightarrow[pH7.8]{AST} L-glutamate+Oxalacetate$$

$$Oxalacetate+NADH \xrightarrow{MDH} Malate+NAD$$

6 Hormonal assay

The other collected serum samples were used to measure testosterone in male rats and oestradiol in female rats, by using Enzyme Linked Immuno Sorbent Assay (ELISA). The assay was conducted at the Camel racing Laboratory, Forensic Science Laboratory, Abu Dhabi, United Arab Emirates. Commercially available testosterone and oestradiol test kits (from

Neogen Corporation, USA) were used. The Kits contained EIA buffer, wash buffer, K-blue substrate, extraction buffer, testosterone or oestradiol enzyme conjugates, testosterone or oestradiol standards and testosterone or oestradiol antibody coated plates (96 wells microplate with anti-testosterone or anti-oestradiol rabbit antibody precoated well).

All glassware were washed and rinsed with deionized water and methanol. Diethyl ether (High Performance Liquid Chromatography-HPLC-grade) was used for extraction of the samples. 200 µl of serum was pipeted into glass test tubes (16 × 10 mm) for serum extraction of testosterone. Diethyl ether (2 ml) was added to the serum and vortex for 30 seconds. The phases were allowed to separate. One ml of the upper diethyl ether was transferred into clean glass test tubes. The solvent was evaporated under a stream of nitrogen. The residue was dissolved in 100 µl of diluted extraction buffer (1:4). The extract was diluted 100 fold by adding 10 µl of the extract to 990 µl of diluted extraction buffer. Aliquots of the latter were assayed in duplicate.

The assay procedure of testosterone was performed automatically on a Dynatech (MR7000) and a BRIO (Basic Radim Immunoassay Operator) machines as follows: 50 µl of the diluted extract was added to each well,

then 50 µl of diluted enzyme conjugate was added (BRIO). The plates were shaked gently (Dynatech MR7000 Shaker) and incubated at room temperature for 1 hour. After incubation, the plates were washed three times with 300 µl diluted wash buffer (10 fold dilution with deionized water). K-blue substrate (150µl) was added to each well. The plates were then shaked gently and incubated at room temperature for 30 minutes. After incubation, the microplates were shacked and read in a microplate reader (Dynatech MR7000 Reader) at 650nm. The concentrations of testosterone in samples were measured against a calibration curve prepared from the stock solution of testosterone provided with the kit. Eight points of the calibration curve were used viz; 0.0, 0.002, 0.004, 0.008, 0.02, 0.04, 0.08, 0.2 ng/ml. The intra assay coefficient of variance for a concentration of 1ng/ml (n= 5) was 22.5%.

The serum concentration of free estradiol was estimated by EIISA Kit (Neogen Corporation, Lexington, KY, USA). Serum estradiol was first extracted as follows: 500µl of serum in a glass test tube were added to 500µl of distilled water. The samples were vortexed for 1 minutes and were poured onto Chemelut (1219-8002, Varian, Harbor City, CA, USA) and were left for 5 minutes. Estradiol was then eluted by 2 x 5 ml of diethyl ether. The diethyl ether was evaporated to dryness under a stream of nitrogen and the

residue was dissolved in 500µl of diluted extraction buffer supplied with the EIISA Kits. Assay of estradiol was followed according to the steps of the kit similar to that of testosterone. The concentrations of estradiol were read from a calibration curve raging from 0 to 2 ng/ml in EIA buffer supplied with the kit. The percent recovery of 1 ng/ml of estradiol in buffer (n = 4) mean ± SEM was 50.9 ± 4.15. The intra assay coefficient of variation for a concentration of 1 ng/ml (n = 4) was 8.15%.

7 Statistical Analysis

Data were analyzed using the Statistical Analysis System program (SAS, 1995). The effect of date pits on rats fed dietary treatments was analyzed with ANOVA using General Lanier Model (GLM) procedure based on a completely randomized design (Steel and Torrie, 1980). Differences between treatments means were tested using the Least Significance Difference (LSD) option. Data were presented as means ± SEM.

RESULTS AND DISSCUSION

RESULTS AND DISSCUSION

Chemical composition of date pits and experimental diets

Tables 2 and 3 show the chemical composition of date pits on dry matter basis. According to the approximate chemical analysis of date pits, the nitrogen free extract (NFE) was calculated by subtracting crude protein (6.0%), crude fiber (13.5%), ether extract (8.0%), and ash (1.0%) from 100 and was found to be 71.5%. All of these values fall within the range of date pits approximate analysis previously reported by many researchers (Kamel et al. 1981; Yousif et al. 1996; Hussein et al. 1998; and Ali et al. 1999).

Because date pits contain between 55-73.7% NFE which is similar to that of grain, many researchers (Rashid & Alwash 1987; Yousif et al. 1996; and Ali et al. 1999) assumed that 55-73.7% NFE in the date pits is mainly starch. However, the result indicated that the chemical analysis of starch content in the date pits was only 3%.

Table 2. Chemical composition of date pits on crude fiber basis[1]

Item	%
Crude protein	6.0
Ether extract	8.0
Crude fiber	13.5
Ash	1.0
NFE[2]	71.5
Starch	3.0
Non-starch	68.5

[1] Each value is the mean of three observations;

[2] Nitrogen- free extract = 100 – (Crude protein + Ether extract + Crude fiber + Ash)

Table 3. Chemical composition of date pits on dietary fiber basis[1]

Item	%
Crude protein	6.0
Ether extract	8.0
Dietary fiber	58.3
Ash	1.0
NFE[2]	26.7
Mannose	20.9
Glucose	2.01
Allose	1.96
Galactose	0.99
Arabinose	0.48
Xylose	0.35
Rhamnose	0.03
Fructose	0.01

[1] Each value is the mean of three observations;

[2] Nitrogen- free extract = 100 – (Crude protein + Ether extract + dietary fiber + Ash)

This study found that NFE was reduced to 26.7 % when analysis was based on dietary fiber. The NFE was calculated by subtracting crude protein (6.0%), dietary fiber (58.3%), ether extract (8.0%), and ash (1.0%) from 100. The high percentage of dietary fiber (58.3%) resulted in reduction of NFE of which 78% is mannose (78% = 20.9/26.7*100). This finding is in agreement with that of Ishrud et al. (2001).

The experimental diets that were fed to rats were formulated to be isonitrogenous (23% CP) and isocaloric (2.8 Mcal/Kg). Crude protein percent was similar in all treatments, but ether extract percent was different in the experimental diets to get isocaloric diets. Thus ether extract percentage were 5.9% in diet 1, 5.7% in diet 2, 8.3% in diet 3 and 4, and 14.0% in diet 5. Crude fiber percentage ranged between 5.3% in diet 4 to 7.6% in diet 1. The ash percent in the five diets range from 6.7% in diet 5 to 8.3% in diet 2 (Table 4).

Table 4. Chemical composition of experimental diets fed to rats (% of DM)

	Diet 1 (Cont.)	Diet 2 (Cont. + vit. E)	Diet 3 (12.5% DP)	Diet 4 (12.5% + vit. E)	Diet 5 (25% DP)
CP	23.0	23.3	23.4	23.8	23.7
E.E	5.9	5.7	8.3	8.3	14.0
CF	7.6	7.1	5.4	5.3	7.2
Ash	7.4	8.3	6.9	6.9	6.7

DM= Dry Matter; CP= Crude Protein; EE= Ether Extract; CF= Crude Fiber.

Feed intake of rats

The effect of dietary treatments on average daily feed intake during each week of the feeding period is shown in Table 5. The higher feed intake in male rats compared to female rats was probably due to the difference in initial body weights. The addition of vitamin E to the control diet (diet 2) significantly increased the daily feed intake of male rats during the first two weeks of the study. However, no further increased in feed intake of male rats was noticed in the last two weeks. The addition of 12.5% date pits (diet 3) or even 25% date pits (diet 5) had no effect on daily feed intake of male rats compared to the control diet (diet 1). Surprisingly, the addition of combined vitamin E with the 12.5% date pits (diet 4) did not show the same trend when vitamin E alone was added to the control diet in male rats. The reason of this is not clear. Regarding female rats, there was an overall no effect of

different dietary treatments on their feed intake.

Table 5. Effect of dietary treatments on average daily feed intake of rats (g/day)

Week	Sex	Diet 1 (Cont.)	Diet 2 (Cont. + vit. E)	Diet 3 (12.5% DP)	Diet 4 (12.5%+vit. E)	Diet 5 (25% DP)	SE
1	Male	52.80^{b}	57.86^{a}	53.70^{b}	53.13^{b}	56.26^{ab}	1.12
	Female	40.66	41.16	40.40	39.56	39.36	1.77
2	Male	59.70^{b}	63.90^{a}	57.80^{b}	57.13^{b}	58.70^{b}	1.12
	Female	41.90	42.86	40.03	39.73	41.53	1.30
3	Male	61.03^{ab}	64.03^{a}	57.80^{b}	58.83^{ab}	60.26^{ab}	1.75
	Female	43.07^{ab}	44.47^{a}	40.27^{ab}	38.60^{b}	40.33^{ab}	1.48
4	Male	60.30^{abc}	62.93^{a}	$57.20b^{bc}$	56.23^{c}	61.06^{ab}	1.40
	Female	41.66	42.26	39.93	39.80	42.96	1.58

$^{a, b, c}$ Means with different superscripts within the same row differ significantly ($P<0.05$)

Furthermore, the effect of dietary treatments on total feed intake and on overall average daily intake of male rats shows that feed intake increased significantly ($P<0.05$) when vitamin E alone was added to the control diet (diet 2) compared to the control diet (diet 1), and was not changed when vitamin E was combined with 12.5% date pits (diet 4). The addition of 25% of date pits had no significant effect on total feed intake and on overall average daily intake of male rats. Also, this data shows that the total feed

intake and overall average daily intake of male rats were higher than that of female rats. In female rats, the dietary treatments had no significant effect on total feed intake and on overall average daily intake. (Table 6)

Our result are in disagreement with previous findings of Jumah et al. (1973) who showed that the feed consumption of chicks receiving date pits in their diet was higher than the feed consumed by birds having no date pits in their diet. Moreover, Vandepopulier et al. (1995) showed that adding 5 to 27% of date pits to the starter diet of broiler supported feed consumption. While, Al-Asgah (1987) reported that total replacement of the bran-barley mixture for young carp by 25, 50, and 75% of date pits meal significantly ($P< 0.05$) reduced daily feed consumption.

Table 6. Effect of dietary treatments on total feed intake (TFI) and on overall average daily intake (OADI) of rats (g/day)

Item	Sex	Diet 1 (Cont.)	Diet 2 (Cont. + vit. E)	Diet 3 (12.5% DP)	Diet 4 (12.5%+vit. E)	Diet 5 (25% DP)	SE
TFI	Male	1697.26^{b}	1804.16^{a}	1642.40^{b}	1633.86^{b}	1715.36^{ab}	32.15
	Female	1212.70	1236.86	1164.70	1143.73	1192.43	42.43
OADI	Male	58.52^{b}	62.21^{a}	56.63^{b}	56.34^{b}	59.15^{ab}	1.10
	Female	41.81	42.65	40.16	39.43	41.11	1.46

^{a,b} Means with different superscripts within the same row differ significantly ($P< 0.05$)

Body weight of rats

The effects of dietary treatments on average weekly weight gain, and on total body weight gain of rats are shown in Tables 7 and 8 respectively. It was found that the addition of vitamin E to the control diet (diet 2) had no significant differences in average weekly weight gain and total body weight gain for both male and female rats. The only exception was in the first week of the study for the male rats. There was a significant increase in the average weekly weight gain of male rats when vitamin E was added to the control diet. The current result is in disagreement with Siegel, et al., (2001). They fed two groups of broilers (A and B) 10 and 300 IU of vitamin E respectively. Their result showed that body weight gains were greater for group B than that of group A. Moreover, Choat, et al., (2000) mentioned that heifers, which were supplemented with high levels of vitamin E, had greater average daily gain from day 14 to 28 of the feeding period. Furthermore, Gill, (1986) stated that supplementing vitamin E at high concentrations in the diet of newly received calves has been shown to improve daily gains during a 28 day of feeding period. On the other hand, Carter, et al., (2000) fed calves different levels of vitamin E for 0, 7, 14 or 28 days during the receiving period. Their data showed that the daily gain in body weight was

not improved by the addition of vitamin E.

These different effects of adding vitamin E to diet of different animals may be due to many factors including different preparations of vitamin E used, different concentration, different diet mixture, and even different animal models. Also, the antioxidant effect of vitamin E and its action as a free radical scavenger can explain most of the effects of vitamin E in animals weight gain. Because of the high fat content in animals fed especially diet 3, 4 and 5, vitamin E requirement is increased as the concentration of polyunsaturated fatty acids (PUFA) in body tissue increases. This relationship is based on the antioxidant properties of vitamin E and the greater susceptibility of PUFA to peroxidation because of the higher proportion of double bonds in the unsaturated carbon chain of the FA. (McDonald et al., 1988; Pond et al., 1995)

The addition of 12.5 % of date pits to the dietary treatment had no significant effect on average weekly weight gain and total body weight gain of both experimental animals compared to the control diet, except week 4 in female rats where there was a significant reduction in body weight gain. Another study, by Al-Azzawi (1960) who found a significant improvement in weight gain when the control diet was substituted with 15 % date pits for barley in poultry rations. Also, Kamel et al., (1981) fed date pits at levels of

5, 10, and 15 % of broiler starter diets for four weeks. Their results indicated that date pits supported chick growth at all dietary levels tested. Vandepopuliere et al., (1995) also found that lower dietary levels of dates, date meat and date pits supported broiler growth. The use of dates and date pits in broiler starter and finisher diets was also found to improve the body weight, total body weight gain, and the efficiency of feed utilization of chicks (Hussein et al., 1998). Also, Ali et al., (1999) reported that feeding male rats date pits for 28 days at levels of 7 and 14 % increased the final body weight significantly. However, Jumah et al., (1973) reported that a broiler diets supplemented with ground date pits at a level of 15 % resulted in a significant depression in the final body weight and growth.

Incorporation of vitamin E with the date pits (diet 4) and adding 25% date pits to the ration (diet 5) had no effect on average weekly weight gain and total body weight gain compared to the control diet for both male and female rats except in week 4, where there was a significant increased in average weekly weight gain for female rats when 25% date pits was added. This is in disagreement with the work of others who reported that adding different level of date pits to animal feed resulted in increased body weight gain (Shakir et al., 1969 [b]; Al-Kinani et al., 1975; Al-Asgah N. A., 1987; and Elgasim et al., 1995).

Table 7. Effect of dietary treatments on average weekly weight gain of rats (g/week)

Week	Sex	Diet 1 (Cont.)	Diet 2 (Cont. + vit. E)	Diet 3 (12.5% DP)	Diet 4 (12.5%+vit. E)	Diet 5 (25% DP)	SE
1	Male	33.27 [b]	39.52 [a]	33.95 [b]	34.22 [b]	36.22 [ab]	1.61
	Female	18.15	19.24	20.35	17.78	18.75	5.04
2	Male	30.50	30.36	30.09	29.23	29.46	2.16
	Female	11.23	12.86	11.96	12.63	12.06	1.33
3	Male	22.87	24.81	24.08	25.06	25.37	2.74
	Female	9.78 [a]	9.74 [a]	4.66 [b]	10.96 [a]	8.84 [ab]	1.75
4	Male	20.80	21.33	22.35	21.73	23.60	3.05
	Female	3.41 [b]	7.53 [ab]	7.19 [ab]	5.01 [ab]	10.46 [a]	2.15

[a, b] Means with different superscripts within the same row differ significantly ($P<0.05$)

Table 8. Effect of dietary treatments on total body weight gain of rats (gram)

Sex	Diet 1 (Cont.)	Diet 2 (Cont. + vit. E)	Diet 3 (12.5% DP)	Diet 4 (12.5%+vit. E)	Diet 5 (25% DP)	SE
Male	102.47	116.02	110.47	110.23	114.64	6.67
Female	42.56	49.36	44.15	57.50	50.11	5.93

Water intake of rats

The effect of dietary treatments on average daily water intake of rats is shown in Table 9. The result of this trial showed that the average daily water intake of male rats was generally higher than that of female rats. There were no significant differences in average daily water intake of female rats for all dietary treatments. However, male rats which received 25% date pits (diet 5) consumed more water than male rats receiving the control diet (diet 1) and was only significant ($P<0.05$) in weeks 2 and 3.

Water intake is usually a highly complicated physiological mechanism affected naturally by thirst center and it is metabolically regulated by many factors including salt intake and output, water loss, high temperature (Pond et al., 1995).

Table 9. Effect of dietary treatments on average daily water intake of rats (ml)

Week	Sex	Diet 1 (Cont.)	Diet 2 (Cont. + vit. E)	Diet 3 (12.5% DP)	Diet 4 (12.5%+vit. E)	Diet 5 (25% DP)	SE
1	Male	88.57^{ab}	93.81^{a}	90.71^{ab}	83.57^{b}	94.76^{a}	2.93
	Female	80.95	79.76	82.61	82.14	80.95	3.75
2	Male	90.95^{bc}	96.90^{ab}	92.14^{abc}	88.81^{c}	98.81^{a}	2.23
	Female	81.66	81.19	79.76	81.19	85.23	3.26
3	Male	95.23^{c}	101.19^{ab}	96.42^{bc}	95.23^{c}	103.10^{a}	1.65
	Female	85.00	84.28	83.81	81.90	83.81	3.23
4	Male	100.00^{ab}	105.00^{a}	99.16^{ab}	94.58^{b}	100.41^{ab}	2.23
	Female	82.08	84.58	83.54	83.95	90.20	3.28

[a, b, c] Means with different superscripts within the same row differ significantly ($P<0.05$)

Vital organs and adipose tissue weight of rats

Table 10, illustrate the effect of dietary treatments on weights of kidney, liver, spleen, and adipose tissue. The kidney weight of male rats was increased significantly ($P<0.05$) only when vitamin E was added to the control diet, and when 25% date pits was added. While in female rats there were no significant differences in kidney weight for all dietary treatments.

The data on liver weights showed a significant increased in male rats, but not in female rats when vitamin E was added to the control diet.

Incorporation of vitamin E with the date pits (diet 4) had no effect on liver weights of male and female rats. The 25% date pits increased significantly the liver weights in female rats but not in male rats compared to the control diets. No significant differences were found for the spleen weight of male and female rats. The current results of spleen weight agree with that of Kamel et al., (1981) who demonstrated that the liver and spleen weights for broilers fed 5, 10, and 15% date pits were not significantly different from control values.

Adipose tissue weights were not significantly affected by any dietary treatment in both male and female rats.

Table 10. Effect of dietary treatments on organs and on adipose tissue weight of rats (gram)

Organ	Sex	Diet 1 (Cont.)	Diet 2 (Cont. + vit. E)	Diet 3 (12.5% DP)	Diet 4 (12.5%+vit. E)	Diet 5 (25% DP)	SE
Kidney	Male	1.61^{b}	1.80^{a}	1.65^{ab}	1.71^{ab}	1.80^{a}	0.05
	Female	1.18	1.19	1.18	1.11	1.22	0.04
Liver	Male	9.80^{b}	11.64^{a}	9.77^{b}	10.94^{ab}	10.79^{ab}	0.50
	Female	6.05^{b}	6.25^{ab}	6.75^{ab}	6.45^{ab}	7.09^{a}	0.30
Spleen	Male	0.491	0.554	0.516	0.568	0.583	0.03
	Female	0.380	0.387	0.393	0.382	0.388	0.01
Adipose tissue	Male	5.34	6.25	6.22	7.24	6.56	0.69
	Female	4.53	3.80	3.28	3.91	4.60	0.46

$^{a, b, c}$ Means with different superscripts within the same row differ significantly ($P<0.05$)

Testes and uterus weights and reproductive hormones levels of rats

The effects of dietary treatments on testes and uterus weights and on reproductive hormone levels of rats are shown in Tables 11 and 12 respectively. The data indicated that dietary treatments had no significant effect in either testicular or uterine weights of rats. This is contrary to the finding of Ali et al., (1999) who reported that the absolute testicular weight

in a rat group fed with 14 % normal date pits was significantly higher ($P< 0.05$) than that of the control groups. However, our data agrees with that of Ali et al., (1999) who found that treatments of rats for 10 days with lyophilized date pits extract or with polar or non-polar fractions did not significantly affect the uterine weight. On the contrary, Elgasim et al., (1995) reported that date pits significantly ($P< 0.01$) increased uterine weight.

Table 11.Effect of dietary treatments on testes and uterus weights of rats (gram, and as percent)

Sex	Diet 1 (Cont.)	Diet 2 (Cont. + vit. E)	Diet 3 (12.5% DP)	Diet 4 (12.5%+vit. E)	Diet 5 (25% DP)	SE
Testes	2.57^{ab} (1.09)	2.76^{a} (1.06)	2.45^{b} (1.01)	2.66^{ab} (1.08)	2.76^{a} (1.09)	0.07
Uterus	0.51^{ab} (0.31)	0.61^{a} (0.36)	0.46^{ab} (0.28)	0.42^{b} (0.26)	0.47^{ab} (0.28)	0.05

$^{a, b}$ Means with different superscripts within the same row differ significantly ($P<0.05$); Number in parentheses represent the percentage weight for testes and uterus which calculated according to the final body weight (= 2.57/final body weight * 100).

The concentration of testosterone was not affected significantly by any of the treatments (Table 12). This data does not agree with that of Ali et al., (1999) who found that testosterone levels in rats given the date pits at levels of 7 and 14 % in feed were about 3 and 5 times that of the control, respectively.

However, the oestradiol concentration in the serum of rats significantly decreased ($P<0.05$) as the percentage of date pits increased (Table 12). Similarly female rats treated for 10 days with lyophilized date pits extract and the polar fraction were found to have a significantly lower oestradiol plasma concentration when compared to the control values. At the same time, the hormone level was not significantly affected when the rats were treated with the non-polar fraction of date pits (Ali et al., 1999). The reduction of oestradiol levels may be due to date pits treatments in rats diets was probably due to an estrogen negative feed back mechanism on the pituitary and/or hypothalamus levels. Elgasim et al. (1995) reported that date pits contain estrogenic like compounds. This estrogenic effect of date pits inhibits the secretion of gonadotrophic hormones from anterior pituitary and in turn suppresses estrogen secretion from ovaries in treated animals (Garner, 2000).

However, the oestradiol concentration in the serum of rats significantly decreased ($P<0.05$) as the percentage of date pits increased (Table 12). Similarly female rats treated for 10 days with lyophilized date pits extract and the polar fraction were found to have a significantly lower oestradiol plasma concentration when compared to the control values. At the same time, the hormone level was not significantly affected when the rats were treated with the non-polar fraction of date pits (Ali et al., 1999). The reduction of oestradiol levels may be due to date pits treatments in rats diets was probably due to an estrogen negative feed back mechanism on the pituitary and/or hypothalamus levels. Elgasim et al. (1995) reported that date pits contain estrogenic like compounds. This estrogenic effect of date pits inhibits the secretion of gonadotrophic hormones from anterior pituitary and in turn suppresses estrogen secretion from ovaries in treated animals (Garner, 2000).

Table 12. Effect of dietary treatments on reproductive hormones level of rats (ng/ml)

	Diet 1 (Cont.)	Diet 2 (Cont. + vit. E)	Diet 3 (12.5% DP)	Diet 4 (12.5%+vit. E)	Diet 5 (25% DP)	SE
Testosterone	4.20	4.33	4.26	4.73	4.38	0.73
Estradiol	0.43^{a}	0.29^{b}	0.22^{bc}	0.19^{bc}	0.13^{c}	0.04

[a, b, c] Means with different superscripts within the same row differ significantly ($P<0.05$)

Blood biochemical parameters of rats

The values of total protein, globulin, albumin, GPT and GOT in rat serum are shown in Table 13. Total protein concentration increased ($P< 0.05$) in male rats serum when vitamin E was combined with the date pits, and also in the group receiving the 25% of date pits. Dietary treatments had no effect on total protein levels in female rats. These results were below the normal range (6.3-8.6 g/dl), except the value of diet 4 for male rats and for the values of diet 3 and 5 for the females rats (GCUEA, 1993). The concentration of globulin in male rats serum showed a significant increase in all dietary treatments except diet 3. However, the globulin concentration in the serum of female rats was not effected by all dietary treatments. The globulin concentration values for both male and female rats were within the normal range (2.4-3.9 g/dl) (GCUEA, 1993). The albumin concentration in

rats serum was not significantly affected by all dietary treatments for both male and female animals. The albumin concentration values for both experimental animals were not within the normal range (3.3-4.9 g/dl) (GCUEA, 1993).There were no significant effect in GPT and GOT levels for male rats for all dietary treatments. However, there were a significant increase ($P<0.05$) in female rats for both GPT and GOT levels when 12.5% date pits were added. Also, GOT level in female rats serum increased significantly when 25% date pits were added to the control diet. The GOT levels were not within normal range (39-92 U/L) for both male and female rats (GCUEA, 1993) while, GPT levels were within the normal range of 17-50 U/L except for diets 1, 2 and 3 for male rats.

Table 13. Effect of dietary treatments on blood chemistry of rats

Item[1]	Sex	Diet 1 (Cont.)	Diet 2 (Cont. + vit. E)	Diet 3 (12.5% DP)	Diet 4 (12.5%+vit. E)	Diet 5 (25% DP)	SE
TP (g/dL)	Male	5.91^{b}	6.26^{ab}	6.03^{ab}	6.30^{a}	6.26^{a}	0.11
	Female	6.16^{ab}	6.17^{ab}	6.43^{a}	5.90^{b}	6.32^{a}	0.13
GLOB (g/dL)	Male	4.73^{c}	5.03^{ab}	4.83^{bc}	5.12^{a}	5.01^{ab}	0.09
	Female	4.87^{ab}	4.87^{ab}	5.10^{a}	4.68^{b}	5.04^{a}	0.11
ALBU (g/dL)	Male	1.17	1.23	1.20	1.17	1.25	0.04
	Female	1.28	1.30	1.33	1.21	1.27	0.04
GPT (U/L)	Male	51.00	51.77	51.33	47.00	49.33	4.75
	Female	33.44^{b}	40.11^{ab}	47.88^{a}	39.66^{ab}	37.55^{ab}	3.69
GOT (U/L)	Male	128.66	100.11	112.66	128.00	114.55	10.78
	Female	100.44^{c}	115.11^{bc}	155.44^{a}	106.22^{c}	136.00^{ab}	9.46

[a, b, c] Means with different superscripts within the same row differ significantly ($P<0.05$); [1] TP= total protein, GLOB = globulin, ALBU = albumin, GPT = glutamic pyruvic transaminase, GOT = glutamic oxalacetic transaminase.

Chemical analysis of rats carcasses

The amount of ash, crude protein, and ether extract in rat carcass are shown in Table 14. It was shown that there were no significant differences in carcass ash content for both male and female rats when compared to their corresponding controls. The dietary treatments had no significant effect in carcass protein content for male and female rats. The only exception was that adding 25% date pits (diet 5), significantly decreased carcass protein content for female rats. This is contrary to the finding of Yousif et al. (1996), who found that protein content of fish was higher in the group fed blood meal diet supplemented with date pits than those fed diets supplemented with dates. Also, the data showed that different dietary treatments had no significant differences in carcass ether extract for both experimental animals except when adding 12.5% date pits and vitamin E to the feed, where a significant increase was observed for male rat. However, Al-Asah (1987), found that total replacement of the bran-barley mixture for young carp by date pits meal significant increased the fat content in cap flesh.

Table 14. Effect of dietary treatments on chemical analysis of rats carcass (gram)

Analysis	Sex	Diet 1 (Cont.)	Diet 2 (Cont. + vit. E)	Diet 3 (12.5% DP)	Diet 4 (12.5%+vit. E)	Diet 5 (25% DP)	SE
Ash	**Male**	9.15	8.16	8.64	9.35	8.86	0.616
	Female	11.74	10.78	11.03	11.97	10.37	0.714
CP	**Male**	57.38	60.32	60.54	59.01	58.22	1.29
	Female	62.26^{a}	62.01^{a}	60.86^{ab}	59.47^{ab}	58.27^{b}	1.30
EE	**Male**	27.28^{b}	28.39^{b}	27.90^{b}	34.76^{a}	31.35^{ab}	1.63
	Female	26.46^{ab}	26.95^{ab}	26.09^{b}	27.52^{ab}	30.10^{a}	1.35

^{a,b} Means with different superscripts within the same row differ significantly ($P<0.05$)

CONCLUSIONS

CONCLUSIONS

According to the approximate chemical analysis of date pits, the NFE was calculated by subtraction and found to be 71.5 %. Our data showed that date pits contain only 3 % starch. However, when chemical analysis was based on dietary fiber, the NFE was found to be 26.7 %. The high percentage of dietary fiber (58.3 %) resulted in a reduction of nitrogen free extract (26.7 %) of which 78 % is mannose.

Because of the low energy content of date pits as a carbohydrate source, the total replacement of high-energy grain by date pits is not recommended. It is clear from this work that although date pits do not contain a high source of carbohydrate, still, date pits were effective in enhancing the growth of the experimental animals even when date pits were added up to 25 %. This growth enhancing effect may be due to other unknown factor(s) in date pits or it could be an effect of high percent of mannose. Further research should focus on the role of mannose in the date pits.

It is hypothesized that from this study that the depression in oestradiol level in female rats was due to the estrogen negative feed back mechanism. The estrogenic effect of date pits inhibited the secretion of gonadotrophic

hormones from anterior pituitary and in turn suppressed estrogen secretion from ovaries in treated animals. Because of the expected effect of date pits on female fertility, it is recommended that feeding date pits to female rats should be done with caution.

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APPENDIX

APPENDIX

Table A: Ingredients of the experimental diets (% of weight)

Ingredients	Diet 1 (Control)	Diet 2 (Cont.+ Vit E)	Diet 3 (12.5% DP)	Diet 4 (12.5% + Vit E)	Diet 5 (25% DP)
Soybean meal	16.00	16.00	15.50	15.50	15.50
Meat meal	7.20	7.20	6.00	6.00	5.30
Sunflower meal	8.00	8.00	5.00	5.00	5.00
Fish meal	5.70	5.70	7.70	7.70	9.80
Corn flower	51.10	51.09	46.50	46.47	27.40
Cellulose	6.60	6.58	-	-	-
Date pits	-	-	12.50	12.50	25.00
Corn oil	2.70	2.70	4.10	4.10	9.30
Ca C_{3}	0.50	0.50	0.50	0.50	0.50
$Ca2(Po4)_3$	0.50	0.50	0.50	0.50	0.50
Premix	0.50	0.50	0.50	0.50	0.50
NaCl	0.20	0.20	0.20	0.20	0.20
Vitamin E	-	0.03	-	0.03	-
Binder	1.00	1.00	1.00	1.00	1.00

Table B. Minerals and Vitamins Premix[1] (g/kg)

Item	Unit	amount
A Stable	IU	12500
D Stable	IU	3000
E Stable	IU	25
K3	MG	2.5
B1	MG	2.5
B2	MG	7
Pantothenic Acid	MG	10
Niacine	MG	40
B6	MG	5
B12	MG	0.02
Folic Acid	MG	1
Choline	MG	600
Biothine	MG	0.06
Vitamin C	MG	100
Manganese	MG	80
Iron	MG	35
Zinc	MG	60
Copper	MG	10
Iodine	MG	1.55
Cobalt	MG	0.26
Selenium	MG	0.15

[1] Source: Emirates Flour and Animal Feed Factory.

Table C. Calculated percentage (%) of protein in the experimental diet

ıgredients	% protein	% feed in Control	% feed in Cont.+ Vit E	% feed in 12.5% DP	% feed in 12.5%DP+E	% feed in 25 % DP	% protein in Control	% protein in Cont.+ Vit E	% protein in 12.5% DP	% protein in 12.5%DP+E	% protein in 25 % DP
bean meal	48.00	16.00	16.00	15.50	15.50	15.50	7.68	7.68	7.44	7.44	7.44
at meal	55.00	7.20	7.20	6.00	6.00	5.30	3.96	3.96	3.30	3.30	2.92
flower meal	27.00	8.00	8.00	5.00	5.00	5.00	2.16	2.16	1.35	1.35	1.35
ı meal	72.00	5.70	5.70	7.70	7.70	9.80	4.10	4.10	5.54	5.54	7.06
n flower	10.00	51.10	51.10	46.50	46.48	27.40	5.11	5.11	4.65	4.65	2.74
ulose	0.00	6.60	6.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
e pits	6.00	0.00	0.00	12.50	12.50	25.00	0.00	0.00	0.75	0.75	1.50
n oil	0.00	2.70	2.70	4.10	4.10	9.30	0.00	0.00	0.00	0.00	0.00
co3	0.00	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00
(PO4)3	0.00	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00
mix	0.00	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00
l	0.00	0.20	0.20	0.20	0.20	0.20	0.00	0.00	0.00	0.00	0.00
E	0.00	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
der		1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00
al amount		100.00	100.00	100.00	100.00	100.00	23.01	23.01	23.03	23.03	23.00

Table D. Calculated percentage (%) of calories in the experimental diets

gredients	Kcal/Kg	% of feed Control	% of feed Cont.+ Vit E	% of feed 12.5% DP	% of feed 12.5%DP+E	% of feed 25 % DP	calorie in control	calorie in Cont.+ Vit E	calorie in 12.5% DP	calorie in 12.5%DP+E	calorie in 25 % DP
ean meal	2530	16.00	16.00	15.50	15.50	15.50	404.80	404.80	392.15	392.15	392.15
t meal	2000	7.20	7.20	6.00	6.00	5.30	144.00	144.00	120.00	120.00	106.00
lower meal	1760	8.00	8.00	5.00	5.00	5.00	140.80	140.80	88.00	88.00	88.00
meal	3190	5.70	5.70	7.70	7.70	9.80	181.83	181.83	245.63	245.63	312.62
flower	3370	51.10	51.10	46.50	46.48	27.40	1722.07	1722.07	1567.05	1566.38	923.38
ulose	0	6.60	6.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
pits	1000	0.00	0.00	12.50	12.50	25.00	0.00	0.00	125.00	125.00	250.00
oil	8000	2.70	2.70	4.10	4.10	9.30	216.00	216.00	328.00	328.00	744.00
co3	0	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00
(PO4)3	0	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00
nix	0	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00
l	0	0.20	0.20	0.20	0.20	0.20	0.00	0.00	0.00	0.00	0.00
E	0	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
der	0	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00
l amount		100.00	100.00	100.00	100.00	100.00	2809.50	2809.50	2865.83	2865.16	2816.15

الغذائية المختلفة. بينما تم الحصول على انخفاض معنوي في تركيز هرمون الأنوثة كلما زادت نسبة تركيز نوى التمر في العليقة. إضافة نوى التمر وفيتامين هـ إلى العليقة لهما نفس التأثير في خفض مستوى هرمون الأنوثة في بلازما الدم لإناث الفئران. ذلك يرجع إلى التأثير الاستروجيني لنوى التمر، والذي قد يؤدى إلى خفض الخصوبة عند إناث الفئران.

ARABIC SUMMARY

الملخص العـربي

داد خمس علائق موحدة في نسبة البروتين (٢٣%) والطاقة (٢,٨ ميغا كالوري/كغم). عليقتان
عات ضابطة (العليقه ١، بدون نوى تمر؛ العليقه ٢، بدون نوى تمر + ٣٠٠ ملغم/كغم فيتامين
العلائق الثلاث الأخرى تحتوى على نوى تمر (العليقه ٣، ١٢,٥ % نوى تمر؛ العليقه ٤،
% نوى تمر + ٣٠٠ ملغم/كجم فيتامين هـ؛ العليقه ٥، ٢٥ % نوى تمر). تم استخدام تسعون
فئران البالغة، ٤٥ إناث؛ ٤٥ ذكور. أعطيت جميع الحيوانات العلائق حتى الشبع لمدة ٢٩ يوم.
اس استهلاك الماء والغذاء يوميا. كما تم قياس الوزن اسبوعيا، بالإضافة إلى بداية ونهاية
بة. في اليوم ٣٠ من التجربة تم قتل الفئران بواسطة التخدير، كما تم جمع عينات الدم من كل
م تحليل تركيز هرمون الذكورة والأنوثة في بلازما الدم، وكذلك تم إجراء التحاليل البيوكيميائية
ما الدم. تم فصل ووزن الأنسجة الدهنية وعدد من الأعضاء الداخلية. أجري لكل من نوى التمر
ائق و الفئران التحاليل الكيميائية وذلك لتقدير كل من البروتين الخام و الألياف الخام والدهن
اد. أظهرت نتائج التحاليل الكيميائية أن المستخلص الخالي الآزوت يساوى ٧١,٥ % منه ٣ %
شا. في حالة التحاليل الكيميائية المعتمدة على الألياف الغير قابله للهضم (dietary fiber) وجد
حتوى التمر من المستخلص خالي الآزوت ٢٦,٧ % والذي يحتوى في مجمله على ٧٨ % من
ز. لا ينصح بالاستبدال الكامل للحبوب المحتوية على كميه عالية من الطاقة بنوى التمر وذلك
ع إلى قلة محتوى نوى التمر من الكربوهيدرات. كما وجد أن تغذية نوى التمر للفئران لغاية ٢٥
مل على تحسين النمو ولم يلاحظ وجود أي تدهور في الوزن. وذلك قد يرجع إلى بعض العوامل
ى في نوى التمر عدا عن محتواها من الكربوهيدرات. لا توجد فروقات معنوية في كمية
لاك الغذاء في جميع حيوانات التجربة. عدا الفروقات المعنوية الموجودة في الذكور عند إضافة
ين هـ على المجموعة الضابطة. كذلك لا توجد فروقات معنوية عند قياس الوزن لجميع حيوانات
ربة. عدا الزيادة المعنوية في العليقه ٢ خلال الأسبوع الأول للذكور، وكذلك في العليقة ٥ خلال
بوع الرابع بالنسبة للإناث. المعاملات الغذائية لم يكن لها أي تأثير معنوي على وزن كل من
ال والأنسجة الدهنية. أما بالنسبة إلى التحاليل البيوكيميائية لبلازما الدم فأن نسبة الجلوبيولين
ت ضمن المعدل الطبيعي. تركيز البروتين الكلى كان ضمن المعدل الطبيعي للذكور عند تغذيتهم
قة الرابعة فقط. وأيضا في العليقة ٣ و ٥ بالنسبة للإناث. العليقة ١ و ٢ و ٣ للذكور كانت ضمن
ل الطبيعي لتركيز انزيم GPT. لم يتأثر تركيز هرمون الذكورة بالنسبة للذكور بالمعاملات

جامعة الإمارات العربية المتحدة
عماده الدراسات العليا

تأثير استخدام نوى التمر على تغذية الفئران من حيث توزيع العناصر الغذائية والخصوبة

رسالة مقدمه من الطالبة
عائشة سـالم عـبيد سـالم الظاهـري

بكالوريوس علوم الغذاء والتغذية- كلية العلوم الزراعية- جامعة الإمارات العربية المتحدة (١٩٩٧)

استكمالا لمتطلبات الحصول على درجة الماجستير في العلوم
(علوم البيئة)

جامعة الإمارات العربية المتحدة
عماده الدراسات العليا
مايو
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