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Food Chemistry 86 (2004) 93–97
Food Chemistry
www.elsevier.com/locate/foodchem

Chemical composition of date pits and reproductive hormonal status of rats fed date pits

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Received 18 July 2003; accepted 18 August 2003

Abstract

Five isonitrogenous (23% CP) and isocaloric (2.8 Mcal/kg) diets with or without date pits were fed to rats to evaluate the effect of feeding date pits on the reproductive hormonal status of male and female rats. Proximate analysis of date pits indicated that the nitrogen-free extract (NFE) was 71.5%, in which 3% was starch. When chemical analysis was based on dietary fibre, the NFE was found to be 26.7% only, in which 20.9% is mannose. 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, this may be due to the estrogenic effect of date pits, which may cause reduction in the fertility of the female rats.

Keywords: Date pits; Nitrogen free extract; Starch; Testosterone; Oestradiol

1. 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° and 39° north (FAO, 1999). Since ancient times, the date palm has been a significant source of food for both human and livestock. Date pits are a by-product of date processing, it is known that the average weight of date pits ranges from 13% to 15% of the date's weight (Hussein, Alhadrami, & Khalil, 1998).

Date pits are used in the feeding of ruminant animals. Crude protein, crude fat, crude fibre and ash range 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 their starch content (55–73.7% NFE) (Al-Asgah, 1987; Al-Azzawi, 1960; Ali, Bashir, & Alhadrami, 1999; Hussein et al., 1998; Rashid & Alawash, 1987; Yousif, Osaman, & Alhadrami, 1996). Other researchers have proposed that date pits have an estrogen-like substance which acts as a phytoestrogen in the body of animals fed date pits (Ali et al., 1999; Elgasim, Alyousef, & Humeida, 1995; Vandepopuliere, Al-Yousef, & Lyons, 1995). However, insufficient data are available on the carbohydrate content and the effect of feeding date pits on the reproductive system. The objectives of this study were to determine the carbohydrate content of date pits and to study the effect of feeding date pits on rat fertility.

2. Materials and methods

2.1. Diet preparation

Five isonitrogenous (23% CP) and isocaloric (2.8 Mcal/kg) diets were prepared; 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% DP; diet 4, 12.5% DP and 300 mg/kg vitamin E; and diet 5,

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doi:10.1016/j.foodchem.2003.08.022

25% DP). Date pits and feed ingredients were ground and used in the experimental diets. The diets were formulated and thoroughly hand-mixed with water. The resulting mixture was pelleted to 11 mm in size. Each experimental diet was separately stored in sealed plastic bags at room temperature until needed.

2.2. Experimental animals

Ninety mature Wistar rats (45 females and 45 males with an average body weight 118 ± 7.5 g, 136 ± 9.6 g, respectively) were fed the experimental diets for 29 days. Each sex group was divided randomly into five groups (9 rats/group). Rats from each group were divided into sub-groups and housed in three plastic cages (3 rats/cage) for the whole period of the study.

2.3. Experimental procedure

All animals were provided with feed and water on an ad libitum basis for 29 consecutive days. Water and feed intakes were measured daily. Body weights were measured weekly and at the beginning and the end of each experimental period. On 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 min. Serum was separated and stored at −18 °C for hormonal analysis. Serum samples were used to determine the concentration of testosterone in male rats and oestradiol hormones in female rats.

2.4. Chemical analysis

Date pit samples were ground to pass a 1 mm sieve and analyzed for crude protein, crude fat, crude fibre, and ash contents (AOAC, 1990). Starch was determined by polarimetry. Starch was hydrolysed to monosaccharides by the action of dilute hydrochloric acid. After starch hydrolysis, glucose solution was measured easily because of its ability to rotate plane-polarized light (TFSR, 1999).

2.5. Hormonal assay

Blood serum samples were used to measure testosterone in male rats and oestradiol in female rats, by using Enzyme Linked Immuno Sorbent Assay (ELISA). Commercially available testosterone and oestradiol test kits (from Neogen Corporation, USA) were used.

2.6. Statistical analysis

Data were analyzed using the Statistical Analysis System programme (SAS, 1995). The effect of date pits on rats fed dietary treatments was analyzed by ANOVA, using the general linear model (GLM) procedure, based on a completely randomized design (Steel & Torrie, 1980). Differences between treatments means were tested using the least significance difference (LSD) option. Data were recorded as means ± SEM.

3. Results and discussion

3.1. Chemical composition of date pits

Table 1 shows the chemical composition of date pits on a dry matter basis. Date pits, contained 6% crude protein, 13.5% crude fibre, 8% ether extract, and 1% ash. Nitrogen free extract (NFE) was calculated and was found to be 71.5%. Results fall within the range of date pits values previously reported by many researchers (Ali et al., 1999; Hussein et al., 1998; Kamel, Diab, Ilian, & Salman, 1981; Yousif et al., 1996).

Rashid and Alawash (1987), Yousif et al. (1996), and Ali et al. (1999) assumed that NFE in the date pits is mainly starch because it amounted to 55–73.7%, which is similar to that of grain. However, our result indicated that the starch content in the date pits was only 3%. Starch content of date pits has not been reported in the literature. This study found that NFE was reduced to 26.7% when analysis was based on dietary fibre. The high percentage of dietary fibre (58.3%) resulted in reduction of NFE in which 78% is mannose (78% = 20.9/

Table 1
Chemical composition of date pits on dry matter basis[a]

Item	%
Chemical composition on crude fibre basis	
Crude protein	6.0
Ether extract	8.0
Crude fibre	13.5
Ash	1.0
NFE[b]	71.5
Starch	3.0
Non-starch	68.5
Chemical composition on dietary fibre basis	
Crude protein	6.0
Ether extract	8.0
Dietary fibre	58.3
Ash	1.0
NFE[b]	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

[a] Each value is the mean of three observations.
[b] Nitrogen-free extract = 100 − (crude protein + ether extract + crude fibre + ash).

26.7×100). This finding is in agreement with that of Ishrud, Zahid, Zhou, and Pan (2001).

3.2. Feed intake of rats

Feed intake was increased significantly ($P < 0.05$) in male rats when vitamin E was added to the control diet (diet 2) but was not affected when vitamin E was added to the diet containing 12.5% date pits (diet 4). The addition of 25% date pits had no significant effect on total feed intake of male rats. Also, these results showed that the total feed intakes of male rats were higher than those of female rats. In female rats, the dietary treatments had no significant effect on total feed intake (Table 2).

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. Jumah, Al-Azzawi, and Al-Hashimi (1973) 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, Vandepopuliere et al. (1995) showed that adding 5–27% of date pits to the starter diet of broiler birds supported feed consumption.

3.3. Body weight of rats

The effects of dietary treatments on total body weight gain of rats are shown in Table 2. The addition of vitamin E to the control diet (diet 2) caused no significant differences in total body weight gain for either both male or female rats. Similar results were reported by Carter, Gill, Confer, Smith, and Ball (2000) who 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. On the other hand, Siegel, Price, Meldrum, Picard, and Geraert (2001) fed two groups (A and B) of broilers diets containing 10 or 300 IU of vitamin E, respectively. Their result showed that body weight gains were greater for group B than that for group A. Moreover, Choat et al. (2000) reported that heifers, which were supplemented with high levels of vitamin E, had greater average daily gains from day 14 to 28 of the feeding period. Furthermore, Gill (1986) reported that, supplementing vitamin E at high concentrations in the diet of newly born calves, improved daily gains during a 28 day feeding period.

These different effects of adding vitamin E to the diets of different animals may be due to many factors, including different preparations of vitamin E used, different concentrations, different diet mixtures, 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 different effects of vitamin E on animal weight gain. Because of the high fat content in animals fed especially diets 3, 4 and 5, vitamin E requirement is increased as the concentration of polyunsaturated fatty acids (PUFAs) 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 (fatty acid) (McDonald, Edwards, & Greenhalgh, 1988; Pond, Church, & Pond, 1995).

The addition of 12.5% of date pits in the dietary treatment had no significant effect on total body weight gain of both experimental animals compared to the control diet. Al-Azzawi (1960) 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) 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 feed utilization efficiency in 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 broiler diets supplemented with ground date pits at a level of 15% resulted in a significant depression of the final body weight and growth.

Incorporation of vitamin E with 12.5% date pits (diet 4) or 25% date pits (diet 5) had no effect on total body

Table 2
Total feed intake and total body weight gain of rats (g) as affected by dietary treatments

Sex	Diet 1 (Cont.)	Diet 2 (Cont. + vitamin E)	Diet 3 (12.5% DP)	Diet 4 (12.5% + vitamin E)	Diet 5 (25% DP)	SE
Total feed intake						
Male	1697.26[b]	1804.16[a]	1642.40[b]	1633.86[b]	1715.36[a,b]	32.15
Female	1212.70	1236.86	1164.70	1143.73	1192.43	42.43
Total body weight gain						
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

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

Table 3
Gonads weights (g) and reproductive hormone levels of rats (%) as affected by dietary treatments

	Diet 1 (Cont.)	Diet 2 (Cont. + vitamin E)	Diet 3 (12.5% DP)	Diet 4 (12.5% + vitamin E)	Diet 5 (25% DP)	SE
Effect of dietary treatments on gonad weights						
Testes	$2.57^{a,b}$ (1.09)	2.76^{a} (1.06)	2.45^{b} (1.01)	$2.66^{a,b}$ (1.08)	2.76^{a} (1.09)	0.07
Uterus	$0.51^{a,b}$ (0.31)	0.61^{a} (0.36)	$0.46^{a,b}$ (0.28)	0.42^{b} (0.26)	$0.47^{a,b}$ (0.28)	0.05
Effect of dietary treatments on reproductive hormones level						
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}	0.73

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

weight gain compared to the control diet for either male or female rats. This is in disagreement with the work of others who reported that adding different levels of date pits to animal feed resulted in increased body weight gain (Al-Asgah, 1987; Al-Kinani & Alwash, 1975; Elgasim et al., 1995; Shakir, Farhan, & Al-Khalisi, 1969).

3.4. Testes and uterus weights and reproductive hormone levels of rats

The effects of dietary treatments on testes, uterus weights and the reproductive hormone levels of rats are shown in Table 3. Results indicate that dietary treatments had no significant effects on 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 of a rat group fed with 14% normal date pits was significantly higher ($P < 0.05$) than that of the control groups. However, our data agree 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 other hand, Elgasim et al. (1995) reported that date pits significantly ($P < 0.01$) increased uterine weight.

The concentration of testosterone was not affected significantly by any of the treatments (Table 3). This result 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. Similarly, Ali et al. (1999) found that giving female rats lyophilized date pits extract and the polar fraction, for 10 days, 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. The reduction of oestradiol levels may be due to date pit treatments in rats diets causing 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 may inhibit the secretion of gonadotrophic hormones from the anterior pituitary, which in turn suppresses estrogen secretion from ovaries in treated animals (Garner & Hafez, 2000).

4. Conclusions

The approximate chemical analysis of date pits showed that the NFE was 71.5%. Our data showed that date pits contain only 3% starch. However, when chemical analysis was based on dietary fibre, the NFE was found to be 26.7%. The high percentage of dietary fibre (58.3%) resulted in a lower NFE value (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, they are still effective in enhancing or maintaining the growth of the experimental animals, even when added up to 25%. This may be due to other unknown factor(s) in date pits or it could be an effect of the high percent of mannose. Further research should focus on the role of mannose in the date pits.

The depression of oestradiol level in female rats may be due to the estrogen negative feed-back mechanism. The estrogenic effect of date pits inhibited the secretion of gonadotrophic hormones from the anterior pituitary and in turn suppressed estrogen secretion from ovaries in treated animals.

Acknowledgements

The authors thank Mr. Omar Salem from Emirates Floor and Feed Mill for suppling the feed ingredients.

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