Egypt. J. of Appl. Sci., 37 (7-8) 2022 *43-57*

STUDYING THE EFFECT OF DIFFERENT METHODS OF QUINOA PREPARATION ON OBESE RATS

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ABSTRACT

Obesity is a metabolic disease that has reached epidemic proportions. It's a risk factor for several co-morbid illnesses including cardiovascular disease, diabetes mellitus, and many types of cancer as well as decreases quality of life .*Chenopodium quinoa* Willd is a grain-like crop traditionally used for nutrition and has health benefits & super food properties. The present study was aimed to investigate the effect of basal diet supplemented with raw, germinated and cooked quinoa on obese rats. The experiment included 35 adult males' albino rats that classified into five groups. The obese rats fed on different quinoa preparation for eight weeks. The quantities of the feed intake and body weight of rats were recorded. Body weight gain percentage and feed efficiency ratio were calculated. At the end of the experiment blood samples were withdrawn for analyzing lipid profile for all studied groups. The most important results showed that the basal diet supplemented with germinated quinoa had the best effect in weight reduction ($P<0.05$) as well as improved indicators of blood samples. Using the different tested methods of preparing quinoa, may be benefit in control obesity.

Key Words: obesity; quinoa; germinated; cooked; total antioxidant capacity; total phenols; lipid profile; rats.

INTRODUCTION

Obesity is a metabolic disease that has reached epidemic proportions. The World Health Organization (WHO) has declared obesity as the largest global chronic health problem in adults which is increasingly turning into a more serious problem than malnutrition **(Frühbeck *et al.*, 2013)**. It has reached epidemic proportions according to the WHO in 2016, whereby,1.9 billion adults were overweight and 600 million of these were considered obese (**WHO, 2016)**.

Obesity results from an interaction between an individual's genetic predisposition to weight gain and environmental influences. Gene discovery in the field of weight regulation and obesity has identified several major single-gene effects resulting in severe and early-onset obesity as well as many more minor genes with more variable effects on weight and fat distribution, including age-of-onset and severity (**Farooqi and O'Rahilly, 2017) .**

Obesity is a risk factor for several of the leading causes of and worsens co-morbid illnesses including cardiovascular disease, diabetes mellitus, and many types of cancer as well as decreases quality of life **(Haire-Joshu and Klein, 2011).**

Quinoa (*Chenopodium quinoa* Willd) is a grain-like crop which is traditionally used for nutrition and sustenance to Andean indigenous cultures for centuries due to claims of health benefits and superfood properties **(Vilcacundo and Hernandez-Ledesma, 2017).** The high nutritional value of quinoa may be attributed to its unique chemical composition as it contains a protein of a high quantity and quality with a balanced essential amino acid pattern. Also, it contains fiber, polyunsaturated fatty acids, saponins, phytosterols, phytoecdysteroids, phenolics, betalains, and glycine betaine (**Graf *et al.*, 2015).**

In addition to high nutritional value and being free of gluten, quinoa was also reported to have many health benefits. It can be used for both children and elderly, for lactose intolerance, those who are suffering from either anemia, obesity, diabetes, ciliac disease or dyslipidemia. It has a high antioxidant and anti-inflammatory potency and can be used as anticancer, neuroprotective and immunomodulatory (**Vilcacundo and Hernandez-Ledesma, 2017).**

MATERIALS AND METHODS:

The biological experiment and the chemical analysis were carried out at the Laboratories of the Regional Center for Food and Feed, Agricultural Research Center, Giza.

Nine kilograms of quinoa seeds (*Chenopodium quinoa* Willd.) were obtained from Seed Institute, Agriculture research center. Seeds were cleaned to remove dirt, stones and other impurities. Stored at ambient temperature conditions (15-34 ^{0}C) till further use. Three kilograms of quinoa seed a third of the total amount were kept for use without any preparation. The rest of the amount of seeds was subjected to two different types of processing, described as follows:

- Germination

The second third of the quantity was used and the seeds were distributed in germination trays on wet laboratory paper and covered with the same wet paper to hydrate the seeds by capillarity. The duration of this germination period was based on the laboratory observation, since in longer periods sprouts overgrow. Seeds stored at room temperature for 72 hr for the germination to take place. In between, water was sprinkled on the wet laboratory paper to maintain seeds moist to achieve good germination. After germination quinoa seeds were dried at 50 °C in a mechanical convection oven to constant weight. Dried sprouts were then milled using a laboratory grinder and the obtained flour was stored in plastic bags in a desiccator at 4

°C until further analysis. This method was carried out according to **Padmashree *et al.*, (2019).**

- Cooking:

Last remaining third of the seeds amount was washed before heat treatment, as it is usually used to reduce the saponin content. In washing, 50 g of grains were exposed to manual rubbing in running water for 15 minutes. For hydration (W + H), washed grains were transferred to a pan of water (1:3 w / v) and kept at 60 ° C for 30 minutes. After processing, grains were ground and stored at 20°C until analysis. This method was described according to **Júlia *et al.*, (2016).**

The analysis of moisture, protein, ash, fat, raw fiber saponin, oxalate and phytic acid in dried quinoa seeds were determined using the standard methods **AOAC, (2012).** The standard methods **FAO/WHO, (1985)** was followed to calculate the gross energy using the following equation: Gross energy = 4 × (Protein % + Carb. %) + 9 × (Fat %). The original total carbohydrate was calculated by difference according to **Merrill and Watt (1973).**

Total antioxidant capacity and total phenolic content (TPC) of the tested samples were determined by the method of **Prieto *et al.*, 1999 and Singleton *et al.*, (1999).** Saponin, phytic acid and oxalates content were determined following by methods **Obadoni & Ochuko 2001 ; Haugh & Lantzch 1983 and Abaza *et al.*, (1968)**, respectively.

Thirty-five adult male rats (Sprague Dawley strain), weighing about 150±5 g was obtained from the Laboratory Animal Colony, Helwan, Egypt.

Diets: Casein, cellulose, choline chloride, D-L methionine, vitamins and minerals constituents were purchased from El-Gomhoriya Pharmaceutical Company, Cairo, Egypt. Starch, soybean oil, and sucrose were obtained from the Egyptian local market.

Chemicals and kits for biochemical analysis were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

Experimental Animal Design:

Induction of obesity: Thirty-five rats were survived in well-ventilated cages under sanitary conditions conforming to health norms and were fed a basal diet **Reeves *et al.*, (1993)** adjusted for one week, and then divided into separate two main groups, the 1st Major group (n=7) continued the basal diet during the experimental period (8 weeks) and served as the negative control group (-ve). The 2nd major group **(High Fat Diet**) (n=28) was fed on the basal diet (with some modifications) for 4 weeks including: Casein 14%, Fiber 5%, Vitamin Mix 1%, Mineral Mix 3.5%, Sucrose 10%, lard 19% + Soybean Oil 1%, L-cystine 0.18%, choline bitartrate 0, 25% and the rest was starch to induced obesity according to **Liu et al., (2004)**. The group (**HFD**) was divided into 4 subgroups (7 rats each) as follow: Subgroup (1) served as a positive control (+ve) group and was fed a high-fat diet until the end of the

experimental period. Subgroups (2 to 4) were fed a high-fat diet supplemented with (dried, germinated, cooked) quinoa at the level of 10%, respectively.

At the end of the experiment rats were fasted overnight then blood samples were collected and centrifuged to obtain serum, which were stored at -20 °C until subsequent biochemical analysis. The total feed intake (FI), body weight (BW) of rat's was recorded. Body weight gain percentage (BWG%) and feed efficiency ratio (FER) were calculated according to **Champman, *et al.*, (1959).**

Biochemical analysis: Total serum cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), and triglycerides (TG) were determined by **Fossati and Principe (1982); Albers *et al.*, (1983) and Jacobs and Vander, (1960)**, respectively. Low-density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) were calculated using the **Fruchart (1982)** equation.

Data were analyzed statistically by SPSS program, one-way ANOVA followed by post hoc multiple were used to make a comparison among different groups **Snedecor and Cochron, (1989)** and the results was communicated as mean± standard Error (SE).

RESULTS AND DISCUSSION:

Table (1) proved that different dried quinoa preparation considered a good source of protein, ash fiber and carbohydrate. It turns out that the best results in proteins were attributed to the germinated quinoa, followed by cooked and then raw (19.1, 16.2 and 14.9) respectively. In the same way, the most effective results of carbohydrate were identified to cooked quinoa, followed by germination one and raw (73.39, 69.79, and 66.99%), respectively. Also, the results of ash, fiber and fat were slightly different according to the 3 ways of quinoa preparing method.

Table 1: Chemical composition of different dried quinoa preparation (%).

Quinoa	protein	Ash	Moisture	Fiber	Fat	Carbohydrate	Gross energy Cal/100g
Raw seeds	14.9	3.86	8.89	4.22	5.36	66.99	375.8
Germinated seeds	19.1	2.66	4.10	5.00	4.35	69.79	394.71
Cooked seeds	16.2	2.99	1.20	5.71	6.22	73.39	414.34

These results united with those reported by **Vilcacundo & Hernandez-Ledesma, (2017) and Wahba *et al.*, (2019)** who found that, carbohydrate and protein content of quinoa were relatively high compared with alternative breakfast cereal like rice and wheat. Moreover, quinoa seeds of high protein quality since it provides with many essential amino acids that meet the necessities for adults as explicit by **WHO, (2010).** This improvement is also thanks to the loss of dry

matter particularly carbohydrate through germination **Uppal and Bains, (2012)** or the reawakening of protein synthesis upon imbibition **Nonogaki *et al.*, (2010)** which could cause the augmented protein content.

Flour given from raw quinoa had higher amounts of crude protein (14.02%), crude fat (5.13%) and total ash (3.83%) **Bhathal *et al.*, (2017**). Also, quinoa seeds provided energy of 371.18 Kcal/100g, fiber and protein (43.08% and 30.62%), respectively, with 20.47% and 10.98% of carbs and fats **Darwish *et al.*, (2020**). Several studies have confirmed the prime quality of protein in quinoa that provides all essential amino acids (**Comino *et al.*, 2013 and Dakhili *et al.*, 2019). Filho *et al.*, (2017**) reported high edibility of quinoa protein. Raw quinoa provides 91.6% proteins, and the amount are often additional raised to 95.3% by heat medical aid **(Ruales *et al.*, 2002).** Due to this high digestibility, the biological worth of quinoa (73%) is like beef (74%) **(Gordillo-Bastidas *et al.*, 2016).**

In addition, quinoa may be a gluten free grain that patients with disorder will safely eat **(Comino *et al.*, 2013).** Lipid content of quinoa has been reportable from 5.3% to 14.5%, characterized by a high degree of unsaturation starting from 70% to 89.4% **(Gordillo-Bastidas *et al.*, 2016).** It contains 8– 13% dietary fiber (**Tanwar *et al.*, 2019).** Quinoa fiber composition varies with totally different growth conditions and genotypes, with insoluble fiber from 10% to 14%, and soluble fiber ranging from 1.3% to 6.1% **(Zhu, 2020).** These results are in harmony with the obtained results in present work.

Results in Table (2) show that the total antioxidant capacity of raw seeds, germinated seeds, and cooked quinoa seeds were (450.44، 998.9 and 699.29 mg/100g AAE) respectively. Also, total phenols concentrations investigated to record (212.5, 362.7 and 287.3 mg/100g GAE/g), in raw seeds, germinated seeds and cooked quinoa seeds individually.

In agreement with a several research which mentioned that germination significantly increased the total phenolic content in quinoa (**Bhinder *et al.*, 2021**). Another report demonstrated that the total phenolic content of quinoa increased by more than 200% after 6 days of germination **(Al-Qabba *et al.*, 2020)**. Polyphenols were significantly increased after 72 h of germination with compared to raw quinoa seeds, whereas fermentation decreased both compounds. Phenolic compounds and antioxidant capacity were enhanced during the germination procedure (**Carciochi, *et al* ., 2016).** The germination method naturally

produced improved ingredients with health-selling antioxidant compounds (**Jia *et al.*, 2019) .**

Table 2: Effect of different methods of quinoa preparation on total antioxidant capacity and total phenols:

Parameter \ Quinoa	Raw seeds	Germinated seeds	Cooked seeds
Total antioxidant capacity mg/100g (ascorbic acid equivalent)	450.44	998.9	699.29
Total phenols mg/100g (gallic acid equivalent)	212.5	362.7	287.3

*** Total antioxidant expressed as ascorbic acid equivalent and total phenols expressed as gallic acid equivalent.**

The results in Table (3) show the anti-nutritional factors of raw seeds, germinated seeds and cooked quinoa seeds were (2.840, 1.272 and 2.676 %) for saponin respectively, and (0.209, 0.130 and 0.187%), for oxalate respectively. In the same way, phytic acid registered these values (1.355, 1.264 and 1.335 %), in raw seeds, germinated seeds and cooked quinoa seeds respectively.

Regarding the effect of different techniques of quinoa preparation on anti-nutritional factors, the obtained results confirmed that cooked quinoa contains the lowest amount followed with germinated seeds, these results agree with work of **Darwish *et al.*, (2020)** who found that germination method decreased the anti- nutritional factors, saponins and phytic acid. Saponins is lowered due to their leaching from quinoa seeds during washing and soaking **(Bhathal *et al.*, 2015).** Phytic acid content significantly lowered due to its break down as source of phosphorus for utilization during germination **(Padmashree *et al.*, 2019).**

Table 3: Effect of different processing on anti-nutritional factors in quinoa seeds.

Quinoa	Saponin	Oxalate	Phytic acid
	%		
Raw seeds	2.840	0.209	1.355
Germinated seeds	1.272	0.130	1.264
Cooked seeds	2.676	0.187	1.335

Some saponins are considered antinutritional because they caused insoluble complexes with some minerals **(Caballero *et al.*, 2003).** Phytic acid is an antinutrient commonly found in plant-source food, which exerts strong chelating activity on minerals or other positively charged molecules. However, germination significantly reduce these antinutritional components and increase the mineral content of quinoa **(Al-Qabba *et al.*, 2020; Darwish *et al.*, 2020 and Bhinder *et al.*, 2021).** In addition, saponins are usually removed from the seed before consumption because of their bitter taste (**Al-Qabba *et al.*, 2020).**

Recently, **Landi *et al.*, (2021)** reported a protein toxin found in quinoa, called, quinoin, which can prevent protein synthesis and was found to provoke cell morphological alternations. In addition, to consume cooked quinoa instead of raw quinoa, because protein toxins are easily denatured at high temperature. Soaking for 12–18 h reduce the levels of proteolytic enzyme inhibitors as well as phytic acid, which are partly or wholly solubilized in soaked water **Kajihausa *et al.*, (2014).**

The results in Table (4) show that IBW was recorded before the induction of obesity, so there was no significant different in the initial body weight of all the experimental groups.

Obese rats fed on different quinoa preparation had significant ($P<0.05$) decrease in FBW, BWG%, FER, as compared to the control (+ve) group. However, fed on germinated quinoa seed significantly ($P<0.05$) lowered the FBW of rats as compared to other obese rat groups. The same trend was observed to the other treated groups. The most effective tested material on FBW was germinated diet followed by cooked and dried quinoa.

The highest reduction in the FBW, BWG% and FER was recorded for group treated with germinated quinoa seed, where the result were recorded (209.70±1.17, -15.77±0.62 and -0.033±0.015) respectively.

Table 4: Effect of different methods of quinoa preparation on initial body weight, final body weight, body weight gain %, feed efficiency ratio and feed intake .

Parameter / Group	IBW (g)	FBW(g)	BWG%	FER	FI (g/d/rat)
Control (-ve)	**250.60±1.20^{a}**	**277.00±2.19^{b}**	**10.53±0.75^{b}**	**0.019±0.014^{b}**	**23.70**
Control (+ve)	**253.40±1.63^{a}**	**312.20±1.39^{a}**	**23.23±1.24^{a}**	**0.034±0.016^{a}**	**29.00**
Dried seeds	**250.20±2.03^{a}**	**235.40±1.56^{c}**	**-5.90±0.48^{c}**	**-0.010±0.009^{c}**	**24.00**
Cooked seeds	**254.00±1.70^{a}**	**226.40±1.80^{d}**	**-10.85±0.91^{d}**	**-0.021±0.019^{d}**	**21.50**
Germinated seeds	**249.00±1.81^{a}**	**209.70±1.17^{e}**	**-15.77±0.62^{e}**	**-0.033±0.015^{e}**	**20.10**

***Results are expressed as mean ± SE.**

***Values in each column which have different letters are significantly different at ($P<0.05$).**

Some findings demonstrated the anti-obesity potential of quinoa polysaccharide **(Cao *et al.*, 2020; Teng *et al.*, 2020 and Ng and Wang, 2021).** High-fat-diet stimulated obese rats complemented with quinoa polysaccharide revealed a lower level of fat accumulation in adipocyte compared with the +ve control group **(Teng *et al.*, 2020).** In addition, the inhibitory effect of phenolic compounds on digestive enzymes helps decrease the absorption of energy **Noratto *et al.*, (2019) . Fotschki *et al.*, (2020)** proved that the body weight of rats fed on diets containing quinoa protein-rich flour was lower than that of the (+ ve) group.

Quinoa seed powder at various levels (10, 20, 30 and 40%) showed a significant development of BWG, FI and FER as compared to the (+ve) group **Wahba *et al.*, (2019).** Quinoa is a very nutritious source because it involves a good balance of carbohydrates, lipid, fiber, amino acids, minerals and vitamins **Vega-Gálvez, (2010)**. Quinoa seeds painting as anti-obesity activities and might be used as a nutritional supplement for treating and inhibiting disorders of obesity **(Hejazi, 2016).** Studies have recounted a significant decrease in BWG in rats fed on high cholesterol-diet supplemented with quinoa **(Halaby *et al.*, 2017 and Alghamdi, 2018),** high-sugar-diet-induced rats **(Lopes, *et al.*, 2018 and Mohamed *et al.*, 2019)** healthful rats **(Fotschki *et al.*, 2020),** and healthy humans **(Pourshahidi *et al.*, 2020).**

The effects of quinoa on weight loss were more evident in the population on a high-fat diet.The capacity mechanisms of quinoa anti-obesity capacity are inhibition of adipocyte differentiation through gene regulation, increase in energy expenditure, and decrease in fat intake. in addition, quinoa can be used as a good source of nutrients for body recovery **(Simnadis *et al.*, 2015 ; Ng and Wang, 2021).**

Lipid profile

The results in Table (5) demonstrated that obese rats had significant ($P<0.05$) increase in values of serum TC, TG, VLDL-c, LDL-c and LDL-c/HDL-c ratio but significant ($P<0.05$) decrease in HDL-c as compared to the control negative group. Groups fed on diets with different preparations demonstrated significant ($P<0.05$) increase in values of HDL-c but lower of other lipids (LDL-c, VLDL-c, TG, TC and LDL-c/HDL-c) as compared to the obese rats fed on high fat diet.

Table 5: Lipid profile in normal and obese rat groups fed on different quinoa preparation after 8 weeks of experiment.

Parameter / Group	TC	TG	VLDL-c	HDL-c	LDL-c	LDL/HDL
	(mg/dl)					
Control (-ve)	104.43±1.39^{d}	87.14±2.13^{d}	17.43±0.42^{d}	61.25±2.02^{ab}	25.75±2.54^{e}	0.428±0.571^{c}
Control (+ve)	214.27±1.87^{a}	148.69±3.37^{a}	29.73±0.67^{a}	29.25±1.28^{d}	155.28±2.17^{a}	5.354±0.278^{a}
Cooked	125.41±1.94^{c}	114.48±1.74^{b}	22.89±0.34^{b}	48.54±2.73^{c}	53.97±4.09^{c}	1.146±0.153^{b}
Dried	150.01±2.01^{b}	118.67±2.11^{b}	23.73±0.42^{b}	56.34±2.06^{b}	69.93±2.03^{b}	1.248±0.073^{b}
Germinated	121.84±1.79^{c}	101.93±1.22^{c}	20.38±0.24^{c}	64.62±2.36^{a}	36.83±2.45^{d}	0.576±0.054^{c}

***Results are expressed as mean ± SE.**

***Values in each column which have different letters are significantly different at ($P<0.05$).**

The best average values for TC, TG, VLDL-C, LDL-C and LDL-C/HDL-C ratios and higher HDL-C values for the group fed on germinated quinoa. On the other hands, cooked were recorded quinoa recorded significant ($P<0.05$) lower values of serum TC and LDL-c compared to dried quinoa. However, dried quinoa demonstrated significant ($P>0.05$) higher values of serum HDL-c than cooked quinoa.

Nonsignificant (P>0.05) differences were found in serum TG, VLDL and LDL/HDL between groups fed on dried or cooked quinoa.

The values of HDL-c in obese rats fed on diets with dried or germinated quinoa did not significantly (P>0.05) differ from that of normal negative control. Germinated quinoa supplementation exerted more effect than that of dried one.

The hypocholesterolemic effect of quinoa may be attributed to its content of saponins **Graf *et al.*, (2015).** Moreover, the fiber content of quinoa may contribute to its hypolipemic effect as the **Navarro-Perez *et al.*, (2017)** found that chronic consumption of quinoa might help reduce the risk of cardiovascular disease**.** The positive effects of quinoa on cardiovascular health was recorded by **Halaby *et al.*, (2017) and Alghamdi, (2018)**. The consumption of quinoa can improve lipid profile, including a significant decrease in TG, LDL-C level, and caused a significant increase in HDL-C level **(Lopes, *et al.*, 2018; Ali, 2019; Mohamed *et al.*, 2019; Wahba *et al.*, 2019; Al-Qabba *et al.*, 2020 and Abdel-Wahhab *et al.*, 2021).** Although the results of various studies could be different, the effects of quinoa consumption on the lipid profile showed a similar trend. Recent evidence supports that saponins have hypocholesterolemic activity **(Marrelli *et al.*, 2016 and Singh *et al.*, 2017).** The physiological effect of consuming quinoa seeds to decrease weight gain and improve lipids profile **Simnadis *et al.*, (2015).** Moreover, **Ali, (2019)** reported that all supplemented groups with quinoa seeds 5 and 10%, had significant increase in HDL-c, while serum, TC and LDL-c were decreased significantly.

Using different methods to preparing quinoa before adding it in diets, may be benefit in fighting obesity. Also, eating quinoa by many ways such as a rice replacement, a hot breakfast cereal, boiled in water for making infant cereal food. As well, the seeds can be popped like popcorn, grind to use as flour, or sprouted. Finally, it could be recommend the use of dried germinated quinoa seeds as a food ingredient in the formulation of valuable functional foods to control obesity.

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دراسة تأثير الطرق المختلفة لإعداد بذور الكنوا على الفئران البدينة

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السمنة هي مرض وبائي ناتج عن خلل في التمثيل الغذائي بالجسم. وهي من أحد الاسباب الرئيسية لتفاقم الأمراض المصاحبة لها كالقلب والأوعية الدموية وداء السكري والعديد من أنواع السرطان. بذور الكنوا عبارة عن محصول يشبه الحبوب يستخدم بشكل تقليدي في التغذية لما له من فوائد صحية وخصائص غذائية ممتازة. تهدف الدراسة الحالية الي التحقق من تأثير تناول الكنوا النيئة والمنبتة والمطهية على الفئران البدينة. شملت التجربة على 35 من ذكور الفئران الألبينو البالغة وتم تقسيمها إلى خمس مجموعات. تغذت الفئران البدينة على الغذائي الاساسي المدعم بالأنواع المختلفة من الكنوا لمدة ثمانية أسابيع. تم تسجيل إجمالي المتناول من الطعام يوميا، وزن الجسم للفئران وحساب نسبة زيادة الوزن وكذلك نسبة كفاءة الغذاء. في نهاية التجربة تم سحب عينات الدم لإجراء تحاليل صورة الدهون. وأظهرت اهم النتائج أن النظام الغذائي الذي يحتوي علي الكنوا المنبتة كان لها أفضل تأثير في إنقاص الوزن بالإضافة إلى تحسين مؤشرات الدم. ويستنتج من ذلك أن استخدام الطرق المختلفة لإعداد الكنوا، قد يكون مفيدا في التحكم في السمنة.