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RESEARCH ARTICLE

Effects of the seed of *phalaris canariensis* and the change of diet on serum lipids in rats

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ABSTRACT

Canary seed is a grassy plant in the family of poaceae, which grains are used almost exclusively to feed birds. With limited information reported but proven bioactive potential, led to study how does the consumption of a canary seed infusion affected rats with a change in a diet on the lipid profile, in an experimental model. The animals were divided into two groups, the first group received a hypercaloric diet (60 days) and the second was maintained on a normocaloric diet. Afterwards the groups were subdivided, one group received a diet change from hypercaloric to normocaloric, the second continued to receive the hypercaloric diet and the third kept a normocaloric diet, half of each group ingested 25% Canary seed infusion and the rest water for 45 days. Triglycerides, total cholesterol, HDL-c and LDL-c were determined. Results showed a statistically significant reduction of 30.08% in triglyceride values in the group with combination of Canary seed infusion with a dietary change, while in cholesterol metabolism there were no significant changes. Thus, the ability of canary seed infusion to reduce serum triglycerides when combined with a dietary change was confirmed.

Keywords: *Phalaris canariensis;* Dyslipidemias; Change of diet; Hypotriglyceridemic; Sprague Dawley

INTRODUCTION

Canary seed (*Phalaris canariensis*) is a grassy plant in the family of poaceae, herbaceous, with a growing cycle and production practices like those of other cereals, such as wheat *(Triticum aestivum* L.) and oats *(Avena sativa* L.). Currently its grains are intended, almost exclusively for the feeding of birds, alone or mixed with other seeds (Cogliatti, 2012), which is grown commercially in various parts of the world (Parodi, 1987). Canary seed is a true cereal with unique composition, such as high protein levels, raw fat, total dietary fiber, and less amounts of starch compared to wheat (El Sayed et al., 1993). Additionally, has non-allergic proteins, as demonstrated in a study which revealed no allergenic cross-reactivity between hairless shelled Canary seed and major allergenic proteins of gluten, soy, peanuts, nuts, sesame, and mustard (Boye et al., 2013). Previously, due to the effects associated with the silice hairs that cover the seed hull, which are highly irritating when they come into contact with human tissues relating it to esophageal cancer (Bhatt et al., 2006), was never intended for human consumption. However, it was proven that consumption of Canary seed infusion in rats at different concentrations had no toxic effect (Magnunson et al., 2014). Recent studies suggest the benefits of canary seed milk as a nutritional supplement (Peña et al., 2019; Dikovsky and Meza, 2020).

Nowadays Canary seed infusions are being used empirically as adjuvant therapy for poor diet related disease such as Metabolic Syndrome, Diabetes Mellitus, Arterial Hypertension, among others. Because the high prevalence and mortality rate related to these diseases, they have become a public health issue worldwide (Bustos, 2003). The presence of sterols are believed to be involved in the bioactive properties of canary seed (Hichem et al., 2017), also after digesting the seed, encrypted peptides are released which may inhibit Dipeptidil dipeptidase IV, angiotensin-converting enzyme activity and increase production of nitric oxide, being these the main target for pharmacological treatment of these diseases (Estrada et al.,

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2013; Valverde et al., 2017; Urbizo-Reyes et al., 2021). By the decreasing in neutrophil affluence and the production of inflammatory cytokines gives canary seed its anti-inflammatory properties (Madrigals and Perez, 2015), also the antioxidant effect attributed to the abundant content of diterpenes, which can protect against oxidative stress and liver damage (Novas et al., 2004; Perez and Madrigales, 2015). Studies have shown that a canary seed aqueous extract possesses a Hypoglycemic as well as a non-toxic effect based on histopathological analyses (Reinoso, 2012). In 2015 Perez et al; Studied the effect of canary seed extract on obese mice, finding that the extract efficiently decreased serum glucose by reducing the insulin resistance.

Information regarding the bioactive effects of cereals such as *Glycine max* (Ponnusha et al., 2011), *Avena sativa* (Ahmad et al., 2014) and *Linum usitatissimum* (Ojeda et al., 2017), is widely reported whereas of Canary seed (*Phalaris canariensis*) scant studies has been performed. Due to the scarce scientific literature available about the health benefits of the consumption of canary seed nutraceutical components, it was proposed in this study to evaluate the effect of this cereal infusion on the rat lipid metabolism under different dietary conditions.

MATERIALS AND METHODS

Ethical considerations

The work was approved by the Standing Committee on Bioethics and Biosecurity of the University of Carabobo (CPBB-UC) and was developed at the Institute of Biomedical Research "Francisco Javier Triana Alonso" of the University of Carabobo, Maracay-Venezuela.

The used Canary seed dry, was purchased from a commercial establishment in the area. The methodology proposed by Reinoso (2012), was used to prepare the infusion (Fig. 2-A).

Experimentation animals

Rats of the Sprague Dawley strain aged between 9 and 10 weeks, with an average weight of 250 g, female and non-blood, were used, obtained from the central biotherium of the Universidad Centro Occidental Lisandro Alvarado (UCLA) located in Barquisimeto-Venezuela. The animals were kept inside the laboratory in separate metal cages, at a constant temperature of 25°C (Figs. 2-B y C).

Experimental design

As the objective of the study was to assess the effect of a bird seed infusion under different conditions, three possible scenarios were simulated. The mice were divided into two groups, one group received a normo caloric diet (DNC) and the second received a hyper caloric diet supplemented with 1% powdered cholesterol (DHC), this phase lasted 60 days. Thereafter, the DNC group was divided into two subgroups of five mice each, one received the birdseed infusion and the other did not. The DHC group was separated into two subgroups, the first continued to receive the hyper caloric

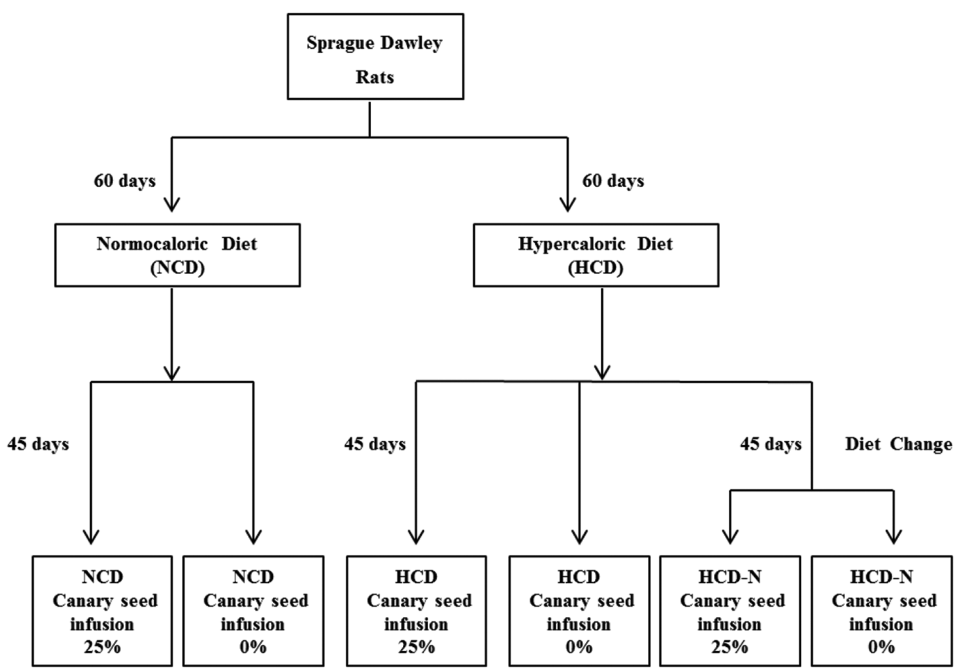

Fig 1. Phase of the study. For 60 days the HCD group, NCD and HCD-N received different diets subsequently subdivided and some received 25% canary seed, others only received a diet change and one subgroup received the most canary seed diet change to 25%, for a period of 45 days.

Fig 2. Images collected during the study. A) Canary seed infusion obtained from cereal. B) Organization of the rats for the experimental design and C) Sprague Dawley rats before sampling.

diet, but five mice received the infusion of birdseed, and the others were given only water. The other group received a diet change to norm caloric (DHC-N), but five mice received the birdseed infusion and the other water. The birdseed infusion was provided *Add libitum* for 45 days (figure 2-B). These three probable conditions were proposed to know the effect of the infusion consumption on three possible scenarios, healthy individuals (DNCs), individuals with dyslipidemias without dietary change (DHC) and dyslipidemia and diet change (DHC-N). The experimental scheme is shown in the following diagram.

For this stage, six treatments were considered in a factorial arrangement 3x2, with three diets: NCD, HCD, and HCD-N and two levels of canary seed infusion: 0 and 25%, applied to five rats each.

Biochemical determinations

Blood samples were collected from the animals using the methodology proposed by Alarcon-Corredor *et al.* (2011). The determination of triglycerides, cholesterol, HDL-c and LDL-c was carried out using commercially available reagents obtained in the locality, following the manufacturer's instructions. Measurements were made on a BECKMAN brand spectrophotometer.

Statistical analysis

A test was conducted in accordance with factorial treatments 2x3 with five repetitions under a completely randomized design. The first factor (Canary seed), consisted of canary seed infusion supplementation from day 46 to day 105, and presented two levels: with supplementation and without supplementation. The second factor (Diet), presented three levels: normocaloric diet for 105 days, hypercaloric diet for 105 days, and change of diet, with hypercaloric diet for the first 45 days, and normocaloric for the remaining 60 days. At the end of the trial, serum concentrations (mg/dL) of triglycerides, total cholesterol, HDL-c and LDL-c were measured.

The normality of the residuals was tested using the Ryan-Joiner test, and the homoscedasticity of the variances of the treatments with the Levene test. The Variance Analysis (ANOVA) was applied to check for differences in the factors included in the model. Multiple comparison tests for the main effects (Canary seed and Diet) were performed using Tukey's honestly significant difference test. The Canary seed*Diet interaction was analyzed graphically. The significance level was set at 5%, so a result was considered statistically significant if $p \leq 0.05$. The data was processed using the Minitab 18.0 statistical program for Windows.

RESULTS AND DISCUSSION

In all of the parameters evaluated, the Ryan-Joiner normality test shown that the residues were normally distributed ($0{,}976 \leq RJ \leq 0{,}992$; $p>0{,}100$) and the Levene test indicated that the treatments were homoscedastic ($0.504 \leq p \leq 0.745$), so the data were analyzed without the need for transformations on them.

Triglycerides

The ANOVA indicated that there were statistically significant differences in the serum concentration of triglycerides when it was classified by canary seed supplementation ($F=8.73$; $p=0.007$) and type of diet received ($F=9.68$; $p=0.001$); In addition, the canary seed*Diet interaction also showed statistically significant differences ($F=5.16$; 0.014), and the effect of the different types of consumption is observed (Fig. 3).

Regarding to the Canary seed supplementation, the Tukey mean comparison test has shown that the serum triglyceride concentration was higher in rats that did not receive canary

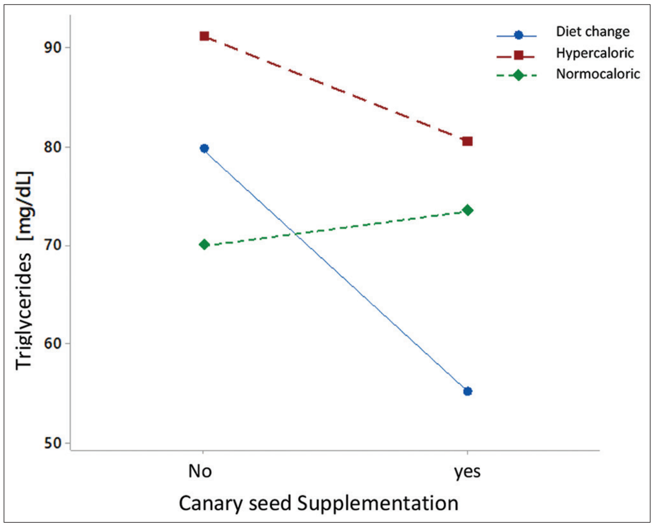

Fig 3. Graph of the Canary seed*Diet interaction for serum triglyceride levels [mg/dL].

seed supplementation (80.29 ± 3.38 mg/dL) compared to the group receiving the canary seed supplement at 25% (69.72 ± 3.61 mg/dL), and for the applied diets, indicated that rats that only received hypercaloric diet had a higher serum triglyceride concentration (85.85 ± 3.22 mg/dL) to those who received only normocaloric diet (71.73 ± 3.59 mg/dL) and dietary change (67.43 ± 4.75 mg/dL), in addition, the latter two diets had a homogeneous response to each other (Table 1).

Regarding to the Canary seed*Diet interaction, in Fig. 1, it can be seen that rats that received hypercaloric diet had the highest levels of triglycerides, although these levels were lower in those who received canary seed supplementation; in addition, rats receiving a normocaloric diet showed similar behavior in both groups, those receiving and those who did not receive canary seed supplementation; Finally, rats that received a diet change showed a much lower triglyceride level in those who received canary seed supplementation, suggesting that the change in diet favors the Hypolipidemic effect of canary seed infusion.

With regard to the hypotriglyceridemic effect observed in this study for rats receiving the change in diet and canary seed (30.08%), it coincides with that obtained by Pérez et al., (2014), who observed a 32% decrease in plasma triglyceride levels, in rats fed a high-fat diet who received 10 weeks of treatment with a Methanol extract from *P. canariensis* and the Rodriguez and Del Aguila, (2015), who achieved a 15.95% reduction in serum triglycerides in Holtzmann rats, which consumed the infusion of canary seed for 30 days

This observed hypotriglyceridemic effect can be caused by the components of this cereal. The canary seed has

Table 1: Arithmetic means and homogeneous Tukey mean groups for serum triglyceride concentration (mg/dL) classified by canary seed supplementation and type of diet received

		Canary Seed 25%		Triglycerides
		YES	NO	(mg/dL)
Type of diet received	DCH-N		X	79.72ᴬ
	DCH-N	X		55.14ᴮ
	NCD		X	69.94ᴬ
	NCD	X		73.50ᴬ
	HCD		X	91.18ᴬ
	HCD	X		80.55ᴬ

Levels within factors with Tukey groups with the same representative letter do not present statistically significant differences to 5%.

in its composition a variety of lipids in representative concentrations (Ben Salah et al., 2017). These include monosaturated (oleic) and polyunsaturated fatty acids (linoleic and linolenic) from the ω family. The presence of these acids may be responsible for the decrease in serum triglycerides observed in this study (see Table 1). Numerous studies have shown the hypotriglyceridemic effect of ω-3 on the metabolism of triglycerides, inhibiting lipogenesis, favoring β-oxidation, increasing the degradation of apo-B and increasing the activity of lipoprotein lipase (Park and Harris, 2003; Jump et al., 2005; Deckelbaum et al., 2006)

Total cholesterol

The ANOVA indicated that there were statistically significant differences in the serum concentration of total cholesterol when it was classified by canary seed supplementation (F=6.86; p=0.004) and type of diet received (F=9.92; p=0.004); however, the canary seed*Diet interaction showed no statistically significant differences (F=0.50; 0.613).

Regarding to canary seed supplementation, The Tukey mean comparison test indicated that the serum concentration of total cholesterol was higher in rats that received canary seed supplementation and for applied diets, indicated that rats that received dietary change had a higher serum total cholesterol concentration than those who received only normocaloric or hypercaloric diets, in addition, the latter two diets had a homogeneous response to each other (Table 2).

HDL-c

The ANOVA indicated that there were no statistically significant differences in the serum concentration of HDL-c when it was classified by canary seed supplementation (F=0.18; p=0.839) and type of diet received (F=1.14; p=0.296); In addition, the Canary seed*Diet interaction also did not show statistically significant differences (F=0.16; 0.854), for this reason the Tukey mean comparison test indicated that the behavior

Table 2: Arithmetic mean and homogeneous Tukey mean groups for serum lipoprotein concentration classified by canary seed supplementation and type of diet received

		Canary seed 25%		Total cholesterol	HDL-c	LDL-c
		YES	NO	(mg/dL)		
Type of diet received	DCH-N		x	190.55^{A}	46.67 ^{A}	129.36^{A}
	DCH-N	x		194.72 ^{A}	47.25^{A}	134.56^{A}
	NCD		x	183.69 ^{A}	46.32 ^{A}	112.90 ^{A}
	NCD	x		179.05 ^{B}	43.06 ^{A}	119.63^{A}
	HCD		x	174.38 ^{A}	45.08 ^{A}	110.25 ^{A}
	HCD	x		179.04 ^{B}	46.75 ^{A}	115.55^{A}

Levels within factors with Tukey groups with equal representative letter do not present statistically significant differences to 5%.

of serum HDL-c levels was homogeneous for both canary seed and diet supplementation (see Table 2).

LDL-c

The ANOVA indicated that there were statistically significant differences in the serum concentration of LDL-c when it was classified by canary seed ($F=16.39$; $p<0.001$) and type of diet received ($F=18.33$; $p<0.001$); however, the Canary seed*Diet interaction showed no statistically significant differences ($F=0.09$; 0.914). In Concern to canary seed supplementation, the Tukey mean comparison test indicated that the serum LDL-c concentration was higher in rats that received canary seed supplementation, and for applied diets, it indicated that rats that received change of diet had a higher serum LDL-c concentration than those who received only normocaloric or hypercaloric diets, in addition, the latter two diets had a homogeneous response with each other (see Table 2). Serum levels of total cholesterol and LDL-c were higher in rats that received canary seed supplementation (Table 2); in the case of HDL-c there were no statistically significant differences when classified by canary seed supplementation, type of diet received and Canary seed*Diet interaction.

By comparing the total cholesterol values observed in this study, with that reported in the literature (Rodríguez and Del Aguila, 2015), it can be noted that there was no match between the two trials. They found a 21.45% decrease in the concentration of this metabolite in Holtzmann rats that consumed the canary seed infusion for 30 days at a rate of 100mg/kg, while in this study there was no variation for cholesterol and LDL-c and HDL-c.

The absence of a hypocholesterolemic effect in this study may be due to several factors. The first may have been associated with the time the animals consumed the infusion. If any bioactive component present in the seed had the property of promoting the excretion of cholesterol (in the form of intestinal metabolites or bile salts) it will require a specific timeframe, since the elimination of this molecule is a slow process (Cofan, 2014). A second possible cause could be the process of making the infusion. According to Ben Salah et al., 2017, this seed has a representative concentration of phytosterols among which stands out the β-sitosterol (molecule associated with a decrease in hypercholesterolemia). The brewing process could lead to a low recovery of these plant sterols in the infusion, which caused the absence of any effect.

CONCLUSION

The hypotriglyceridemic effect of a change of diet and *the ad libitum* consumption of infusion of the canary seed at 25% was confirmed, canary seed consumption did not alter normal values. Regarding to cholesterol and lipoproteins responsible for its transport, no decreasing effect was demonstrated on serum concentrations.

CONTRIBUTION FROM THE AUTHORS

L.O. and N.N-M., design of essays and writing. L.P-Y., statistical analysis, Y.D., J.T., R.O., R.V., F.M., experimental development. F.M. English Language Review.

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