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Impact of consumption of cooked red and black *Chenopodium quinoa* Willd. over blood lipids, oxidative stress, and blood glucose levels in hypertension-induced rats

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Abstract

Background and objectives

Hypertension is associated with the overproduction of free radicals, generating oxidative stress, which could contribute to the lipid peroxidation, altering the blood lipid levels, and to the development of diseases such as diabetes. The aim of this work was to evaluate the bioactivity of cooked red and black quinoa over blood lipids, oxidative stress and glucose levels in hypertension-induced rats by the supply of the drug N (ω)-nitro-L-arginine methyl ester (L-NAME).

Findings

The consumption of red quinoa increased significantly ($p < .05$) the levels of high-density lipoprotein (HDL). In addition, the quinoa consumption, regardless of the variety, not only increased the activity of antioxidant enzymes (superoxide dismutase and catalase), but also reduced blood glucose levels. The total phenolic compounds and total flavonoids content were higher in red than in black quinoa while this latter obtained better values for the total antioxidant activity.

Conclusions

The presence of bioactive compounds in quinoa could be the responsible for its capacity to improve the HDL levels, the in vivo antioxidant activity and the levels of fasting blood glucose in hypertension-induced rats.

Significance and novelty

Findings from this study could promote the consumption of quinoa, which seems to be a good source in the development of functional foods, in order to take advantages of its bioactivities.

Keywords: Antioxidant activity, *Chenopodium quinoa* Willd., glucose sugar levels, hypertension, blood lipids, oxidative stress.

1. INTRODUCTION

Hypertension affects approximately 25% of the adult population in the world, and it is predicted to reach 29% by 2025 (Ngo et al., 2014). Despite being a controllable disease, this pathology is associated with atherosclerosis, changes in the lipid profile, and cerebrovascular accidents (Chaudhari & Patil, 2019; de Castro & Sato, 2015). The decrease in nitric oxide leads to the reduction of its antioxidant potential, promoting oxidative stress and hypertension, since nitric oxide acts as a vasodilator (Korsager Larsen & Matchkov, 2016). In addition, the oxidative stress has been related to the lipid peroxidation and the complications of diabetes because of the development of endothelial dysfunction (Dasgupta & Klein, 2014).

In that sense, a wide amount of antioxidants obtained from foodstuff has been isolated in order to tackle the effects of the oxidative stress. Quinoa (*Chenopodium quinoa* Willd.) has been recognized as a potential source of antioxidants because of its high amount of flavonoids, phenolic compounds, and other bioactive compounds (Ayyash, Johnson, Liu, Al-Mheiri, & Abushelaibi, 2018; Pellegrini et al., 2018), especially the colored varieties after a previous cooking (Bernuy-Osorio, Riveros-Lizana, Villanueva-Espinoza, Suárez-Cunza, & Vílchez-Perales, 2018). However, up to our knowledge, there is little information about the effect of cooked quinoa consumption on the pathologies related to hypertension. Therefore, the aim of this study was to investigate the bioactivity of two varieties of cooked quinoa over the blood lipids, oxidative stress (by measuring the activity of two antioxidant enzymes), and glucose levels in N (ω)-nitro-L-arginine methyl ester (L-NAME)-induced hypertensive rats.

2. MATERIALS AND METHODS

2.1. Materials and reagents

Quinoa Red Pasankalla (RQ) and Black Collana (BQ) were acquired from a local market in La Molina (Lima, Peru). Reagents such as 2,2′-azinobis-3-ethylbenzotiazilone-6-sulphonic acid (ABTS) radicals, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, captopril, 2 N Folin-Ciocalteu, and L-NAME were purchased from Merck (Darmstadt, Germany) and Sigma Chemicals Co. (St. Louis, MO).

2.2. Experimental animals

Thirty male Holtzman rats weighing 180 ± 20 grams were provided by the "Laboratorio de Análisis Biológico" of the Universidad Nacional Agraria La Molina (UNALM). The animals were kept in individual cages. All the animals were maintained under standard environmental conditions: controlled temperature (22–26°C), light–dark cycles of 12 hours and relative humidity

of 70%. Water and a solid standard diet were offered *ad libitum*. The chemical composition of the standard diet (dry weight basis, d.w) was: 20% protein, 3.47% fiber and 7.48% fat (Planta Piloto de Alimentos Balanceados of the UNALM, Peru). After two weeks of growing and acclimatization, rats were randomly assigned to the treatments. All the procedures in this study were in accordance with the law 11794-2000: "Ley Peruana de protección a los animales domésticos y a los animales silvestres mantenidos en cautiverio" and the guidelines of the "Centro Interdisciplinario de Estudios en Bioética de la Universidad de Chile para el cuidado y uso de animales de laboratorio" was followed (Martínez, Rodriguez, Fernando, & Stepke, 2007). Functional analyses were performed with animals under ketamine (60 mg/kg, intraperitoneal) and xylazine (15 mg/kg, intraperitoneal) anesthesia, and all efforts were made to minimize suffering.

2.3. Quinoa characterization

The impurities were removed from quinoa and a manually washing was performed. For cooking, the grains were added in boiling distilled water (proportion 1:3) and were drained after 20 minutes (Dini, Tenore, & Dini, 2010). The cooked RQ and BQ together were dried in a hot-air tunnel (relative humidity of 22% and air flow rate of 2.5 m/s) at 70°C for three hours to be ground (Model A11 Basic, IKA, USA) until a final particle size of <100 μm. Percentage of total moisture and total protein was evaluated in the cooked quinoa flours obtained. Total antioxidant capacity (AC), total phenolic compounds (TPC), and total flavonoids (TF) were performed in both types of cooked quinoa, with a previous methanolic extraction. All the results were expressed in cooked quinoa flour dry weight basis.

2.4. Methanolic extraction

One g of cooked quinoa flour was mixed with 22.5 mL of 80% methanol solution. The mixture was homogenized under constant agitation by a magnetic stirrer (Model M6, CAT, Germany) in darkness for 1 hour at 140 rpm, protected from light. Then, it was centrifuged at 1,878 *g* for 10 minutes at 4°C (Model Rotofix 32A, Hettich, Germany) and the supernatant was reserved. The meal obtained was subjected to a second extraction with 10 mL of 80% methanol solution. This mixture was homogenized during 30 min (at the same conditions reported in the previous homogenization). Then, it was centrifuged at 1,878 *g* for 10 minutes at 4°C. The supernatant obtained was mixed with the previous one and then the methanol was rotary evaporated (Model R-300, BUCHI, Germany) in order to obtain the final extract.

2.5. Experimental protocol and procedures

The animals were randomly assigned to the five treatments (n = six animals per treatment): Control diet without quinoa (T_1), L-NAME + cooked RQ (T_2), L-NAME + cooked BQ (T_3), L-NAME + reference drug captopril (T_4), and L-NAME alone (T_5), for 21 consecutive days. The L-NAME dose administered (40 mg/kg) assured the induction to hypertension since a 20-40% blood pressure increase have been reported previously (Ramírez, Palacios, & Gutiérrez, 2006). This drug was supplied by oral gavage and rats in T_2, T_3 and T_5 also received intragastrically 1 mL of 0.9% of NaCl by oral gavage while rats in T_4 were administered by 1 mL of a captopril (10 mg/kg) solution. The supply of L-NAME during the 21 days of treatments was carried out to avoid the regression of hypertension by L-NAME withdraw (Márquez-Ramírez et al., 2018). Rats in the control received 1 mL of 0.9% of NaCl as replacement of L-NAME and another 1 mL as replacement of captopril. A 30% of cooked quinoa flour in the diet was included in the corresponding treatments (T_2 and T_3), as showed in Table 1, where quinoa composition was taken from previous research (Vilcacundo & Hernández-Ledesma, 2017). At the end of the 21 days, rats were euthanized and the blood was collected by cardiac puncture. The serum was separated for further experiments and the liver was isolated, weighed, and homogenized. Briefly, 1 g of liver was washed with 0.9% of NaCl and taken to the glass homogenizer type Potter-Elvehjem with Teflon plunger. A 10% homogenate was prepared in 50 mM of sodium phosphate buffer at pH 7.4. This whole procedure was worked in an ice bath at 4°C. Subsequently, a centrifugation at 1,790 *g* for 15 minutes at 4°C (Model Rotofix 32A, Hettich, Germany) was performed and the supernatant was reserved for the analysis of soluble protein and antioxidant enzymes activity analyzes.

2.6. Analytical determinations

2.6.1. Total protein

The total protein percent of quinoa was determined by Kjeldhal method, following the AOAC guidelines (AOAC, 2007) and using a conversion factor of 5.85.

2.6.2. Total phenolic compounds (TPC) and total flavonoids (TF)

The TPC were determined with the Folin-Ciocalteu reagent using gallic acid as standard (Singleton & Rossi, 1965). Absorbance was measured at 755 nm and TPC were expressed as mg gallic acid equivalents per 100 g of quinoa (mg GAE / 100 g).

TF were determined by using the aluminum ion colorimetric method (Chang, Yang, Wen, & Chern, 2002), using quercetin as standard. Absorbance was measured at 415 nm and the TF were expressed as mg of quercetin equivalents per 100 g of quinoa (mg CE / 100 g).

147 *2.6.3. Total antioxidant activity*

148 The ABTS decolorization assay was used with slight modifications (Arnao, Cano, & Acosta,
149 2001). The stock ABTS solution included 7.4 mM ABTS and 2.6 mM potassium persulphate
150 solution. The working solution was prepared by mixing the two stock preparations in a 1:1
151 proportion and then performing a 12 h reaction at room temperature in darkness. Then, 1 mL of
152 ABTS solution and 60 mL methanol were mixed to reach 1.1 ± 0.02 units of absorbance at
153 734 nm. Fresh ABTS solution was prepared for each assay. Later, 150 µL of quinoa methanolic
154 extract was mixed with 2850 µL of ABTS. After 1.5 h of reaction under darkness at 20°C, the
155 decrease in absorbance at 734 nm was calculated. The antioxidant activity was calculated as µmol
156 Trolox Equivalents per g of quinoa (µmol TE)/ g).

157 *2.6.4. Protein analyses*

158 The content of soluble proteins of quinoa and homogenized rats liver were determined
159 according to the literature (Lowry, Rosebrough, Farr, & Randall, 1951).

160 *2.6.5. Determination of blood lipids*

161 Triglycerides (TG) and high-density lipoproteins (HDL) were evaluated through commercial
162 measurement kits, which are based on the reflection photometry methodology, as proposed by the
163 literature (Ma et al., 2016).

164 *2.6.6. Determination of superoxide dismutase (SOD) activity*

165 The activity of SOD was measured by inhibiting the autooxidation of pyrogallol at alkaline
166 pH with the formation of the superoxide radical at 420 nm. This reaction follows a linear kinetics
167 and is inhibited by the enzyme SOD (Marklund & Marklund, 1974). The results were expressed as
168 enzyme unit (U) / mg protein.

169 *2.6.7. Determination of catalase activity*

170 The catalase activity assay was based on the decomposition of H_2O_2 into water, with a
171 decrease in absorbance at 240 nm. The reaction was performed by adding 50 mM of phosphate
172 buffer at pH 7.0 to the samples and measuring the absorbance reduction for 3 minutes (Aebi,
173 1984). The activity of catalase was expressed as U / mg protein.

174 *2.6.8. Determination of blood glucose levels*

175 The blood glucose level was evaluated through commercial measurement kits, as reported
176 previously (Bagul et al., 2012).

177 ***2.7. Statistical analysis***

The data represent the mean and standard deviation for six rats per group, and of three independent experiments (n=3), in the case of the quinoa characterization analysis. Data were analyzed by one-way ANOVA ($p < .05$) followed by the post-hoc Duncan test ($p < .05$) using Statgraphics Centurion XVI (StatPoint Inc., Rockville, MD, USA).

3. RESULTS AND DISCUSSION

3.1. Quinoa characterization

The obtained cooked RQ and BQ presented a total protein content of 14.16 ± 0.11% and 14.92 ± 0.05% (dry weight basis, d.w), respectively ($p > .05$). These values are higher than reported by Pellegrini et al. (2008), but lower than reported previously in white quinoa (Repo-Carrasco-Valencia & Serna, 2011). In addition, it is important to mention that cooking increases the digestibility of quinoa protein by reducing the content of trypsin inhibitors and favoring the denaturation of this macronutrient (Bax et al., 2013; Graf et al., 2015).

3.1.1. Total phenolic compounds and total flavonoids

As shown in Figure 1, RQ obtained a higher content of both, TPC and TF (99.02 ± 2.50 mg GAE/100g and 126.49 ± 8.89 mg CE/100g, respectively) than BQ (76.02 ± 7.84 mg GAE/100g and 90.92 ± 14.79 mg CE/100g, respectively), agreeing with a previous work in which RQ obtained a higher soluble phenols content (Starzyńska-Janiszewska, Stodolak, Duliński, Mickowska, & Sabat, 2017). In addition, quinoa was reported to present higher amounts of TF than TPC (Pellegrini et al., 2018), as found in this research. Cooked quinoa has been previously reported to present 13.37% more TPC than raw quinoa (Nickel, Spanier, Botelho, Gularte, & Helbig, 2016). However, it is important to mention that TPC are released in the cooking water since this process increases the bioactive compound accessibility. Therefore, it is advisable to cook quinoa only with the necessary amount of water to reduce the loss of not only TPC, but also flavonoids and the antioxidant capacity (Teixeira-Guedes, Oppolzer, Barros, & Pereira-Wilson, 2019). Regarding the type of phenolic compounds found in quinoa, the majority are under the free form. However, the presence of bound phenolic compounds was also reported (Abderrahim et al., 2015). Within the TPC presented in quinoa, 4-hydroxybenzoic, gallic acid and quercetin are the most representative (Pellegrini et al., 2018).

3.1.2. Total and specific antioxidant activity

Conversely to obtained in TPC and TF, it is appreciated from Table 2 that BQ presented a higher total antioxidant capacity (AC) than RQ, as reported in a previous study (Pellegrini et al., 2018). The total AC is attributed mainly to the TPC and TF present in quinoa (Liu et al., 2020). In

addition, the AC of RQ is related to its content of betalains (Escribano et al., 2017). It is important to mention that cooking is able to increase the AC of quinoa and beans, obtaining a 5.90 and 14.36% higher value than raw quinoa seeds when using the radical DPPH and FRAP, respectively (Nickel et al., 2016; Teixeira-Guedes et al., 2019). Likewise, it was mentioned that TPC are presented in higher proportion in colored varieties of quinoa, and these metabolites are the major responsible factors for the total AC (Bernuy-Osorio et al., 2018).

Nevertheless, in this study, a negative correlation was found between the total AC and TPC [r = -0.9224 (p = 0.0088)], agreeing with some reports in literature which have attributed the AC to others compounds apart from TPC, such as certain vitamins (Orsavová, Hlaváčová, Mlček, Snopek, & Mišurcová, 2019). On the other hand, when TPC were removed from wheat protein, there was a remaining AC, which leads to thinking that there are other important compounds involved in the above mentioned bioactivity (Gammoh et al., 2018). In this context, a positive and high correlation was found between the total AC and the soluble proteins content of both quinoa varieties [r = 0.8780 (p = 0.0214)], which indicates a high association degree. The specific AC in protein is also reported in Table 2, BQ obtaining the highest value, but not significant statistically.

By themselves, proteins such as casein, β-lactoglobulin and lactoferrin have presented AC due to their capacity to inactivate the ROS; however, the high molecular weight they have does not allow them to cross the cell membrane, being unable to act biologically. Therefore, a further hydrolysis or fermentation is required in order to generate the bioactive peptides from native proteins (Ayyash et al., 2018; Samaranayaka & Li-Chan, 2011; Starzyńska-Janiszewska et al., 2017). Regarding the release of the bioactive peptides, a temperature of 70°C (used in this study to cook both quinoa varieties) is ideal to generate them since it leads the protein denaturation (Bax et al., 2013). Furthermore, at an *in vitro* level, the AC of quinoa proteins not only increased when bioactive peptides were generated due to the action of the gastrointestinal enzymes but also maintained their bioactivity during the whole digestion process (Vilcacundo, Miralles, Carrillo, & Hernández-Ledesma, 2018).

Besides the content of TPC, TF and bioactive peptides released through quinoa cooking, carotenoids, tocopherols and tocotrienols were reported to be possible contributors to the AC of this Andean cereal (Tang et al., 2015). Subsequently of the characterization of RQ and BQ, biological assays were carried out in order to evaluate the bioactivity of said Andean grain over blood lipids, oxidative stress, and blood glucose levels in hypertension-induced rats.

3.2. Biological assays

3.2.1. Determination of blood lipids

Figure 2 shows the effect over the content of TG and HDL in hypertension-induced rats. The increase of blood pressure was observed to not have a significant effect on the concentration of both TG and HDL after the 3 weeks evaluated , but it is highly recommended to control the lipid profile during hypertension since this condition was reported to raise TG concentration (Chaudhari & Patil, 2019), and to produce atherosclerosis after a 4-week supply of L-NAME (40 mg/kg) (Ramírez et al., 2006). However, the quinoa consumption, regardless the variety, increased the serum content of TG (T_1 used as reference), but not statistically significant. Despite the increase of TG generated by quinoa consumption, these values did not exceed maximum allowable limits in rats, 108.11 mg/dL (Ihedioha, Noel-Uneke, & Ihedioha, 2013). The lowest TG value after the 3 weeks of assessment was obtained when captopril drug was supplied (T_5), suggesting a relationship between the antihypertensive treatment and the concentration of TG. Regarding this topic, an association between captopril and resveratrol (a phenolic compound) over the improvement of the lipid profile was reported in mice after 8 months of treatment (de Almeida Pinheiro et al., 2017).

On the other hand, HDL is a specific lipid transport circuit that reverses cholesterol transport by removing it from tissues (Maranhão, Casela Filho, Sigal, Chagas, & da Luz, 2018). Furthermore, HDL levels have been recognized to present an inverse relationship with the cardiovascular risk since it could influence the inflammation, angiogenesis and glucose homeostasis (Nicholls & Nelson, 2019). Figure 2 depicts that the RQ consumption improved the HDL values in 42.32%, this value being higher than the normal range reported in rats (Ihedioha et al., 2013). Quinoa consumption was reported to inhibit the decrease of HDL caused by a 5-week supply of fructose (Paśko, Zagrodzki, Bartoń, Chłopicka, & Gorinstein, 2010). In addition, quinoa presents a 10% of fiber, whose intake has an inverse relationship with the development of cardiovascular diseases, promotes satiety, and reduces the cholesterol and other lipids absorption (Graf et al., 2015).

3.2.2. Determination of superoxide dismutase and catalase activity

Previous research has reported that hypertension is related to an increase in oxidative stress. An elevation in the concentration of superoxide anion and hydrogen peroxide, as well as a decrease in the synthesis of nitric oxide and lower bioavailability of endogenous antioxidants was found in hypertensive patients (Touyz, 2004). Thus, Table 3 shows the activity of SOD and catalase, after the 3-week administration of treatments in rats induced to arterial hypertension.

SOD is the enzyme that catalyzes the dissociation of the free radical O_2^- in water and hydrogen peroxide. The activity of this enzyme increased by 73.07% and 72.08% when RQ and BQ, respectively, were supplied to hypertension-induced rats. A similar result was found after the consumption of *Kalanchoe pinnata* (Bopda et al., 2014), due to the flavonoids that plant presents.

Regarding catalase activity, rats fed with BQ presented 96.88% more activity than the T_1, this value being higher than the RQ group, but only numerically ($p > .05$). In addition, catalase enzyme activity was increased after 4-week of administration of protocacheic acid (a type of phenolic acid), to rats previously induced to hypertension. That compound is responsible for inhibiting the pathway of the hypertensor nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in the endothelium, which is an enzyme that plays an important role in the generation of ROS (Safaeian, Emami, Hajhashemi, & Haghighatian, 2018). Furthermore, resveratrol has been associated to the increase of both SOD and catalase activity in rats induced to oxidative stress (Fu et al., 2018). In the present study, a correlation of 88.24% was found between the activities of the two enzymes studied [$r = 0.8824$ ($p = 0.0003$)].

It is worth noting that the hypertension induction did not have a significant effect on the activity of both enzymes. However, a previous 28-day study showed a reduction of the SOD and catalase activities due to the administration of L-NAME. Also, the above mentioned study found that the reduction of the activity was slowed down when taurine or an antihypertensive drug were supplied to hypertensive-induced rats (Adedara et al., 2018). According to Table 3, when captopril was given to rats, a non-significant increase of both enzymes are reported as well as when L-NAME alone was supplied. This could be explained since the induction to hypertension produces a higher production of free radicals and therefore, the activity of the antioxidant systems is increased as part of our endogenous defense mechanism (Sarmadi & Ismail, 2010); however, if that system is not helped by external sources of antioxidants, the oxidative stress cannot be controlled. That is why those rats fed with quinoa after the hypertension induction obtained higher levels of activity of the antioxidant enzymes SOD and catalase.

Likewise, quinoa flakes have been related to the increase of the activity of glutation (antioxidant endogenous peptide) while this behavior was not present after the consumption of corn flakes (De Carvalho et al., 2014). It was reported that quinoa brings a moderate protection of antioxidant enzymes in the heart, kidney, plasma, and pancreas (Paśko et al., 2010) since this Andean cereal contains fiber, vitamins (especially tocopherols and carotenoids), phenolic compounds, and phytosterols (De Carvalho et al., 2014).

306 *3.2.3. Determination of blood glucose levels*

307 Figure 3 shows the effect of quinoa intake over the blood glucose in hypertension-induced
308 rats. The lowest blood glucose values were found in those rats fed with quinoa, regardless the
309 variety, reaching a concentration of 101.33 and 96.17 mg/dL, when RQ and BQ were supplied,
310 respectively. Quinoa has several compounds that may be related to its hypoglycemic effect. First
311 of all, fiber could modulate the postprandial insulin response because that compound generates
312 satiety, and some epidemiological studies have found an inverse relationship between dietary fiber
313 consumption and the development of type II diabetes (Graf et al., 2015). In addition, certain
314 polyhydroxylated steroids have been attributed to this bioactivity (Graf et al., 2014) while the
315 content of TPC and tocopherols were the main responsible for decreasing the blood glucose levels
316 when a daily consumption of a cereal bar quinoa-based was carried out (Paśko et al., 2010).
317 Furthermore, the quinoa protein content was related to the low-glycemic index since this
318 macronutrient slows digestion and emptying of the stomach (Shin, Ingram, McGill, & Poppitt,
319 2013). On the other hand, when cooked legume *Lupinus mutabilis* was supplied in the diet, a
320 lower blood glucose level was obtained in comparison to the raw version (Baldeón, Castro,
321 Villacrés, Narváez, & Fornasini, 2012), highlighting the importance of this process. Although
322 cooking would increase the digestibility of starch in cereals, leading to the blood glucose levels
323 elevation, this process was also found to enhance the TPC (Nickel et al., 2016), which may be one
324 of the compounds implied in the quinoa hypoglycemic activity, as mentioned above. In addition,
325 the starch present in this Andean cereal is lower than in wheat and rice while a significant higher
326 protein content was reported (Vilcacundo & Hernández-Ledesma, 2017). During cooking, some
327 bioactive peptides could be released as result of protein denaturation (Bax et al., 2013). Recently,
328 quinoa peptides have been assessed to inhibit the angiotensin-I converting enzyme (Chirinos,
329 Pedreschi, Velásquez-Sánchez, Aguilar-Galvez, & Campos, 2020), which is strongly related to
330 hypertension. During this pathology, a correlation between the levels of angiotensin-II (a potent
331 vasoconstrictor) and insulin resistance was reported, relating diabetes with hypertension (Min et
332 al., 2017). Also, bioactive peptides were related to managing endothelial dysfunction and its
333 complications, insulin resistance being one of them, especially in hypertensive patients
334 (Chakrabarti & Wu, 2016).

335 Despite a positive effect of antihypertensive drugs over blood glucose was found (de Almeida
336 Pinheiro et al., 2017), the present study did not show that trend since there was no significant
337 decrease of that parameter when captopril was supplied, according to Figure 3.

CONCLUSIONS

The present study found that the cooked BQ had a higher AC than RQ while the latter presented more TPC and TF at an *in vitro* level. Regarding blood lipids, a significant increase in HDL levels after the consumption of RQ was reported; however, further research should be done to determine the mechanism involved in this bioactivity. In addition, the consumption of RQ and BQ contributes to the reduction of blood glucose levels, as well as to the reduction of oxidative damage by increasing the activity of SOD and catalase when an induction to hypertension was carried out.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1. Chemical composition of diet with quinoa included

Ingredients	Standard diet* (%)	Quinoa* (%)	
Protein	20.00	14.90	
Fiber	3.47	9.35	
Fat	7.48	6.45	
Proportion	**70%**	**30%**	**Final proportion (%)**
Protein	14.00	4.47	18.47
Fiber	2.43	2.81	5.24
Fat	5.24	1.94	7.18

*Values expressed in dry weight basis (d.w)

548 **Table 2. Total and specific antioxidant activity of cooked red and black quinoa**

549

Parameters	Red quinoa	Black quinoa
Total antioxidant activity (μmol TE/g)*	$21.10^{b} \pm 0.24$	$24.96^{a} \pm 0.92$
Specific antioxidant activity (μmol TE/g protein)*	$3{,}075.74^{a} \pm 124.53$	$3{,}283.67^{a} \pm 101.95$

550

551 *Mean values and standard deviations are presented. Different letters within the same row
552 indicate significant differences, according to the Duncan test ($p < .05$).

553 **Table 3. Antioxidant enzymes activity in hypertension-induced rats**

554

Group	Superoxide dismutase (U/mg protein)	Catalase (U/mg protein)
Control	$19.02^{b} \pm 1.37$	$0.52^{b} \pm 0.20$
L-NAME+red quinoa	$32.92^{a} \pm 6.21$	$0.89^{ab} \pm 0.15$
L-NAME+black quinoa	$32.73^{a} \pm 9.46$	$1.03^{a} \pm 0.32$
L-NAME+captopril	$21.36^{b} \pm 2.85$	$0.66^{b} \pm 0.08$
L-NAME	$20.09^{b} \pm 2.07$	$0.73^{ab} \pm 0.02$

555

556 *Mean values and standard deviations are presented. Different letters within the same column
557 indicate significant differences, according to the Duncan test ($p < .05$)
558 Abbreviation: L-NAME, N(ω)-nitro-L-arginine methyl ester

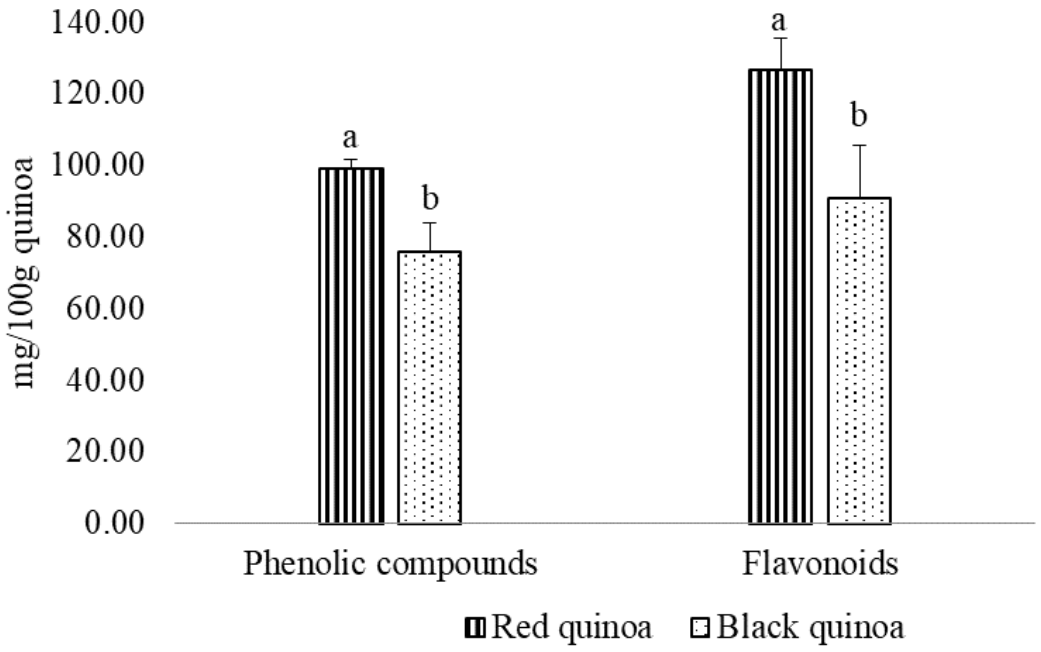
Figure Legends

Figure 1. Total phenolic compounds (TPC) and total flavonoids (TF) in red and black cooked quinoa

Figure 2. Effect of treatments over the blood lipids in rats

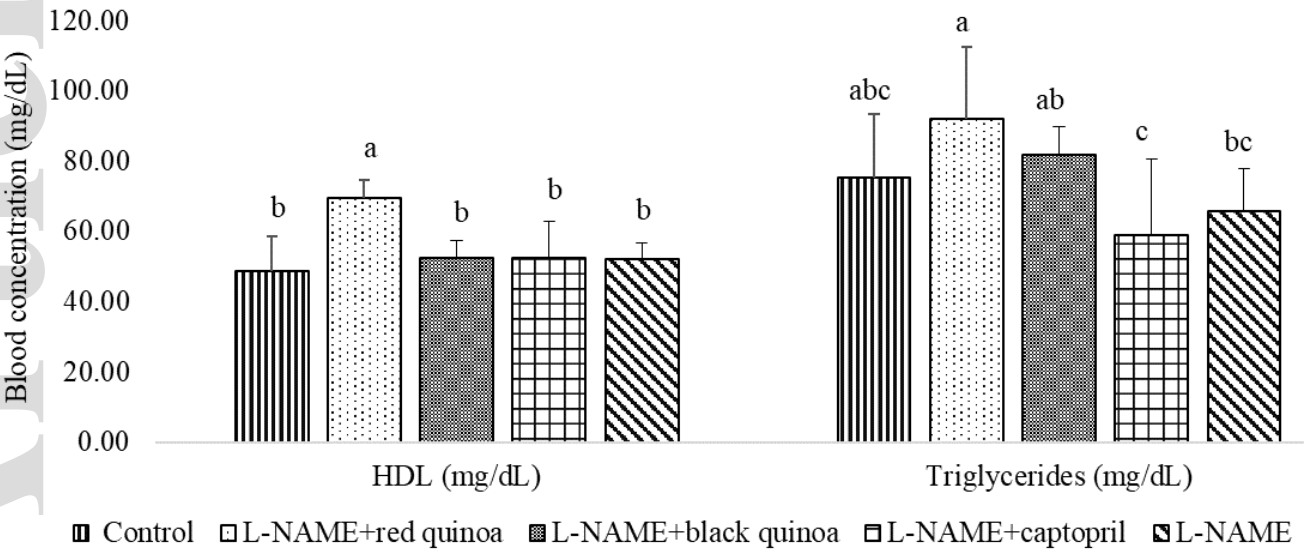
Figure 3. Effect of treatments over the blood glucose levels in hypertension-induced rats

Figure 1



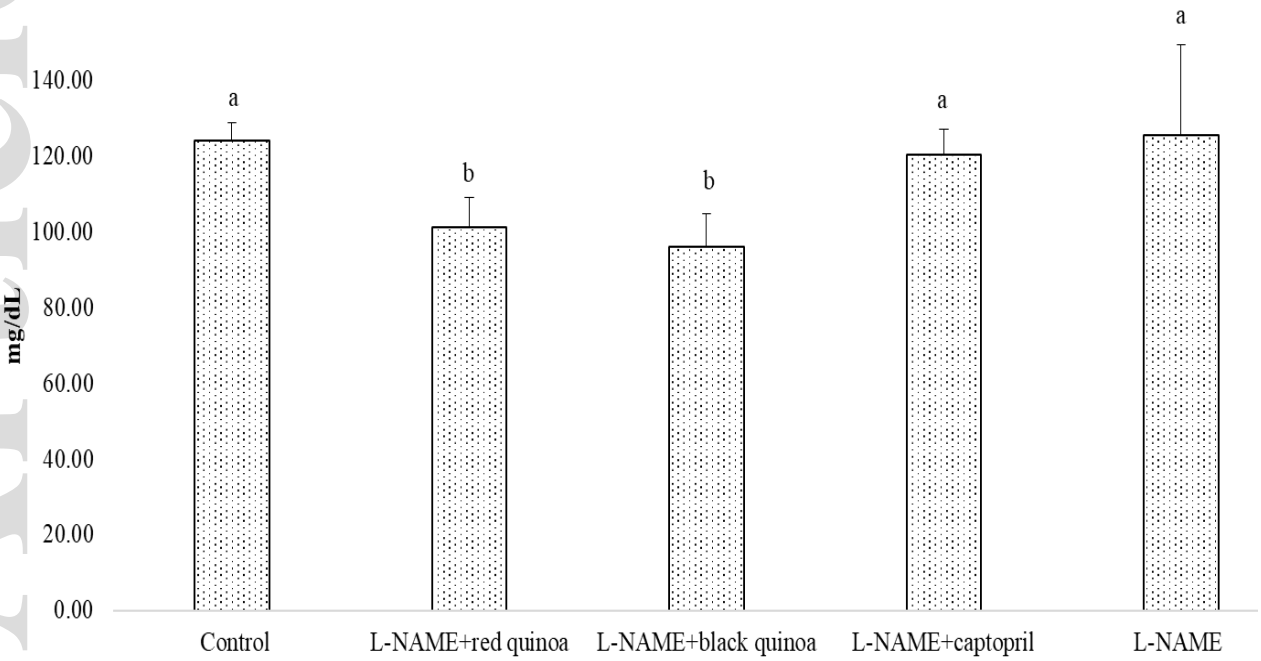
Different letters indicate significant differences, according to the Duncan test ($p < .05$)

Figure 2



Different letters indicate significant differences, according to the Duncan test ($p < .05$). Abbreviation: L-NAME, N(ω)-nitro-L-arginine methyl ester

Figure 3



Different letters indicate significant differences, according to the Duncan test ($p < .05$)

Abbreviation: L-NAME, N(ω)-nitro-L-arginine methyl ester