

RICE AND BEANS TARGETED FOR BIOFORTIFICATION COMBINED WITH HIGH CAROTENOID CONTENT CROPS (SWEET POTATO AND PUMPKIN) REGULATE TRANSCRIPTIONAL MECHANISMS INCREASING IRON BIOAVAILABILITY

ARROZ E FEIJÃO ALVO PARA A BIOFORTIFICAÇÃO COMBINADOS COM CULTURAS COM ALTO CONTEÚDO DE CAROTENOIDES (BATATA-DOCE E ABÓBORA) REGULAM MECANISMOS TRANSCRICIONAIS AUMENTANDO A BIODISPONIBILIDADE DO FERRO

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RESUMO - O objetivo do estudo foi avaliar o efeito de combinações de feijão e arroz, alvos para biofortificação, com culturas com alto conteúdo de carotenoides (batata doce e abóbora) sobre a biodisponibilidade de ferro, expressão gênica de proteínas envolvidas no metabolismo do ferro e o efeito antioxidante. Os grupos testes apresentaram valores semelhantes de eficiência de regeneração da hemoglobina, sendo maior do que o controle, exceto para o grupo Feijão BRS Pontal. Os animais que receberam uma dose adicional de carotenoides pró-vitamina A apresentaram maior expressão de DMT-1 e DcytB. O grupo alimentado apenas com feijão BRS Pontal apresentou menor ($p < 0,05$) capacidade antioxidante total do plasma. A combinação de arroz e feijão, alvos para a biofortificação, com culturas com elevado conteúdo de pró-formadores de vitamina A (abóbora e batata doce) aumentou a expressão gênica de proteínas envolvidas no metabolismo de ferro, favorecendo a sua biodisponibilidade e a capacidade antioxidante.

Palavras-chave: expressão genética, capacidade antioxidante, ferro, batata-doce, abóbora.

ABSTRACT - The objective of the study was to evaluate the effect of food combinations of beans and rice, target for biofortification, with high carotenoids content crops (sweet potato and pumpkin) on the bioavailability of iron, gene expression of proteins involved in iron metabolism, and the antioxidant effect. The test groups showed similar levels of hemoglobin regeneration efficiency, which were higher than the control, except for the group BRS Pontal bean. The animals that received an additional dose of pro-vitamin A carotenoid showed enhanced expression of DMT-1 and DcytB. The group fed only BRS Pontal bean showed lower ($p < 0.05$) plasma total antioxidant capacity. The combination of rice and beans target for biofortification with high pro-vitamin A carotenoid content crops (pumpkin and sweet potato) increased gene expression of proteins involved in iron metabolism, favoring its bioavailability and antioxidant capacity.

Keywords: gene expression, antioxidant capacity, iron, sweet potato, pumpkin.

INTRODUCTION

Iron (Fe) deficiency is the most prevalent nutrient deficiency worldwide affecting about 40% of the world population, particularly women and children in developing countries (MUTHAYYA et al., 2013). Global efforts to reduce the incidence of this nutritional deficiency have been directed at increasing the consumption of micronutrient supplementation through fortification and biofortification of food. The biofortification of staple crops is a new public health approach to control vitamin A deficiency, iron and zinc in poor countries (BOUIS et al., 2011).

The biofortification program with iron has not been successful in increasing the bioavailability of iron to desired levels, which may impact the nutritional status in animals and in humans (VAZ-TOSTES et al., 2015). The association with vitamin A sources can be an alternative to improve the effectiveness of biofortification on human health. Thus, the objective of the study was to evaluate the effect of food combinations of beans and rice, target for biofortification, with high carotenoids content crops (sweet potato and pumpkin) on the bioavailability of iron, gene expression of proteins involved in iron metabolism, and the antioxidant effect.

METHOD

Sample

Staple food crops from Brazilian Biofortification programme were evaluated: common beans BRS Pontal (high Fe content); polished rice Chorinho (source of zinc); pumpkin Duchesne and sweet potato (high pro-vitamin A carotenoid content). Cultivars were developed and supplied by the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Brazil.

Staple food crop flours preparation

The beans were cooked in three replicates in a conventional pressure cooker and dried in an air oven for 17 hours at 60°C (RAMÍREZ-CÁRDENAS et al., 2008). The rice was cooked in three replicates and dried in an air oven for 17 hours at 60°C. The pumpkin and sweet potato were peeled and sliced on a multiprocessor and dried in an air oven for 6 hours at 60 °C. All foods were ground by mill 090 CFT at 3000 rpm, sieved (600 mesh screen) and stored at -12 °C.

Animals and diets

Bioavailability of the iron was measured by the depletion/repletion method. Forty-nine male Wistar rats were placed in seven experimental groups (n=7): Pontal bean (PB); rice + Pontal bean (R+BP); Pontal bean + sweet potato (PB+SP); Pontal bean + pumpkin (PB+P); Pontal bean + rice + sweet potato (PB+R+SP); Pontal bean + rice + sweet potato (PB+R+SP); positive control (Ferrous Sulfate).

The quantity of pumpkin and sweet potato added in the experimental diets was calculated to provide 4.5 mg of vitamin A per kg of diet. This value was based on the conversion of milligrams of vitamin A per gram of body weight in the study by Mwanri et al. (2000) in which anemic children between 9 and 12 years old were supplemented with 1.5 mg of vitamin A per day to assist in the recovery of iron status.

The hemoglobin gain (HG), hemoglobin regeneration efficiency (HRE) and biological relative value of HRE were evaluated.

Determination of gene expression of proteins involved in iron metabolism

The expression of the protein divalent metal carrier (DMT-1), duodenal cytochrome b (DcytB), ferroportin and hephaestin mRNA from duodenum, ferritin and transferrin mRNA from liver were analyzed by reverse transcriptase polymerase chain reaction (RT-PCR). The relative expression levels of mRNA were normalized by the endogenous control glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Antioxidant capacity total plasma

The total plasma antioxidant capacity (TAC) was measured by the colorimetric method with the Sigma kit (Sigma-Aldrich). The concentration of antioxidants in plasma was expressed as mM Trolox equivalent.

Statistical Analysis

Experimental treatments were arranged in a completely randomized design (n=6). The results were analyzed by ANOVA ($\alpha=5\%$). The Dunnett test was used to compare each test group with control group. Newman-Keuls test was used to compare the averages of all experimental groups.

RESULTS AND DISCUSSION

It was observed that the groups fed diets containing combination of Pontal bean and high pro-vitamin A carotenoids crops (PB+P; PB+SP; PB+R+P; PB+R+SP) had HG similar to the positive control group that receiving ferrous sulfate as a iron source ($p\geq 0.05$). The vitamin A can act in iron mobilization from stocks of organic tissues, favoring the availability of this mineral for hematopoiesis and hemoglobin synthesis, increasing the HG (SEMBA; BLOEM, 2002).

The animals that received an additional dose of pro-vitamin A carotenoid showed enhanced expression of DMT-1 and DcytB, which also may have assisted in increasing the uptake and incorporation of iron in the hemoglobin resulting in higher GHb values. Vitamin A has been associated with the regulation of the synthesis of DMT-1 proteins and ferroportin (OATES, 2006).

The PB group showed lower HG than other groups and, when the iron consumption was corrected by iron incorporated into hemoglobin, the PB group remained the lowest value of HRE. The poor performance of this group may be associated with a lower mRNA expression of DMT-1 and ferroportin, proteins that are necessary for the absorption of iron and outsourcing enterocytes (BRASSE-LAGNEL et al., 2011)

The animals fed diets containing only the Pontal bean had less HG and HRE than control and other test groups ($p<0.05$). When the Pontal bean was associated with the rice (PB+R) the HG remained lower than the control, but the HRE was higher ($p<0.05$) and similar to the control test group ($p\geq 0.05$). This result may be related to the fact that this combination provided an increased amount of sulfur amino acids such as cysteine, which has been reported to promote the bioavailability of iron (HE et al., 2013).

The group fed only Pontal bean showed lower ($p<0.05$) plasma TAC. When the Pontal bean was combined with rice, the TAC was similar ($p\geq 0.05$) to the groups supplemented with carotenoids.

CONCLUSION

The combination of rice and beans target for biofortification with high pro-vitamin A carotenoid content crops (pumpkin and sweet potato) increased gene expression of proteins involved in iron metabolism, favoring its bioavailability and antioxidant capacity.

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