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Recuperation levels of hemoglobin (Hb) in Wistar rats with fermented beet by *Lactobacillus sporogenes*

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Abstract

The effect of a diet based on fermented beets and oranges with *Lactobacillus sporogenes* in male Wistar rats with induced anemia was evaluated. No significant difference was found after 120-hour treatment with respect to a diet without fermented beets and oranges. However, the recovery with diet alone consisting of fermented low iron and protein shows a possible beneficial effect in the recovery from anemia.

Key Words: anemia, animal assay, *Beta vulgaris* L., hemoglobin, iron.

Recuperación de niveles de hemoglobina (Hb) en ratas Wistar con betabel fermentado por *Lactobacillus sporogenes*

Resumen

El efecto de una dieta de fermentado de *Lactobacillus sporogenes* con betabel y naranja fue evaluado en ratas Wistar con anemia inducida. No se encontraron diferencias significativas a las 120 horas de recuperación con respecto a una dieta sin fermentado. Sin embargo, la recuperación con una dieta únicamente con fermentado con deficiencia de hierro y proteínas muestra un posible efecto benéfico en la recuperación de anemia.

Palabras Clave: anemia, ensayo animal, *Beta vulgaris* L., hemoglobina, hierro.

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Introduction

Anemia is a common world health issue (Brabin *et al*., 2001). World Health Organization defines anemia as an acquired condition caused by low hemoglobin levels as a consequence of the deficiency from one or more essential nutrients (Stoltzfus, 2001). When hemoglobin levels decrease, life quality is affected (Shander *et al*., 2011). As a consequence of this health issue, it is considered important to develop new strategies that might help reduce the disorder incidence index or ultimately aid in the recovery of those already affected.

A strategy may be the consumption of the beet (*Beta vulgaris* L.). This vegetable is one of the most important, worldwide. It is recognized in increasing exercise-running performance, management of hypertension, antiradical, antimicrobial and cytotoxic activities, as well as by its hepatoprotective and antidiabetic potential (Bolkent *et al*., 2000; Váli *et al.,* 2007; Kapadia *et al*., 2011; Murphy *et al*., 2012; Siervo *et al*., 2013; Vulić *et al*., 2013). According to its pigment, beet is used for hematological disorders. However, there is little evidence about of its anti-anemic properties (Jaiswal *et al*., 2014), so this study is designed to investigate, the effects of a diet based on fermented beet and orange with *Lactobacillus sporogenes* on male Wistar rats with anemia.

Materials and Methods

Forty male Wistar rats (*Rattus norvegicus*), weighing 200-300 g and 4-6 weeks old, were used in this study in the Department of Research and Posgrade in Foods of the University of Sonora, in accordance with the "Guide for the care and use of laboratory animals" (National Research Council U.S.). Firstly, rats were separated into labeled as G1, G2 and G3 (Table I) three groups, with each group, containing ten rats. Each group was put into individual boxes with water and ad libitum. Every two days during the morning, blood was collected from their tails by drop in volumes of approximately 1-2 mL. Hemoglobin (Hb) was measured for levels Hb≤10, which is considered as anemia. In addition, a control group (G0) of rats without anemia induction was formed. The non-anemic group was fed ad libitum. Hb was determined by the HemoCue Hb 201® system, which consists in a portable photometer and microcubes. It measures total Hb in blood capillary and venous (microliters). The diet provided for the control group was a croquette (LABDIET 5008-Formula Diet). G1, G2 and G3 were fed with croquette during the blood extraction (Table I). When rats were diagnosed with anemia, the diet for the G1 group continued to consist of the croquette, while the diet for G2 group was changed to fermented beet and the G3 group was changed to a mixture of the 50% ground croquette and 50% (Table I). The orange juice was added to the beets to be homogenized mechanically through a blender (Osterizer®) during 30 s. The final formula was beet stem (20% w/v), beet tuber (40% p/v), orange juice (5% v/v) and 200 mL of water (35% v/v). A 10^6 of *Lactobacillus sporogenes* inoculum was added to the formula, and incubated (35 °C) to be fermented; the fermentation was stopped when pH ≤5 was reached. Moisture, ashes, proteins, and lipids were determined by Official Methods of Analysis of AOAC INTERNATIONAL, and iron content by ortho-phenanthroline method (Arango *et al*., 2012). Data from the proximate analysis and "experimental rat assays" were used for the statistical analysis. Tukey multiple comparison tests were used to determine differences between means. Statistics were determined using JMP 5.0.1 software (SAS Institute Inc. USA).

Results

Fermented beet was obtained by incubation on day 5, resulting in a pH of 4.68±0.12. Table II shows proximate analysis results of fermented and croquette-fermented diets. Referring to the croquette, results corresponded to amounts reported on the commercial label. Anemia was induced in 10 days after the blood extraction stage. The control group (G0) did not develop anemia.

Hb levels were around 8.5-10.0 g/dL and were kept at least by two consecutive measures. Next, the recuperation process started with the diets. Table III shows Hb levels according to different hours and recuperation diets. No significant differences were found between the groups during 120 hours of recuperation.

Discussion

In regard to iron and protein concentration, the fermented was the diet with lower levels; this data was of special interest because even the fermented group did not have a balanced formula with the croquette. It did not indicate a significant difference in recuperation. However, it did indicate a beneficial possible effect of fermented in Hb.

Group	Diet before anemia induction	Diet after anemia induction
G0 (control group without anemia induction)	croquette	croquette
G1	croquette	croquette
G2	croquette	fermented beet
G3	croquette	croquette (50%) and fermented beet (50%)

Table I. Diets before and after anemia induced in Wistar rats.

	Croquette	Fermented
Moisture (%)	9.85±0.36^{B}	76.20±0.12^{A}
Protein (%)	17.56±1.32^{A}	0.56±0.13^{B}
Lipid (%)	22.90±5.51^{A}	15.30±1.31^{A}
Ash (%)	7.91±0.30^{A}	0.40±0.01^{B}
Iron (mg Kg^{-1})	285.13±16^{A}	7.60±3.48^{B}

Averages for three replicas and standard deviation. Letters indicate statistic groups in lines.

Table II. Diets proximal analysis made to Wistar rats.

Time (hours)	Croquette	Fermented	Croquette-Fermented
12	9.14±1.28^{B}	9.10±2.01^{B}	12.02±1.18^{A}
24	10.24±1.03^{A}	9.82±1.74^{A}	10.87±0.75^{A}
48	11.22±1.48^{A}	10.7±0.1^{A}	11.53±0.45^{A}
96	11.47±1.92^{A}	12.00±1.56^{A}	14.15±0.35^{A}
120	11.35±2.05^{A}	13.44±0.88^{A}	14.77±1.11^{A}

Averages of eight experimental subjects and standard deviation. Letter indicate statistic groups in lines.

Table III. Hemoglobin levels on anemia recuperation during 120 hours.

Benefits of fermented beet and non-fermented beet have been reported and indicated that they could have a significant incidence on anemia recuperation. Saccharose, glucose, fructose, citric acid, fumaric acid, malic acid, polyphenols, vitamin C, betalains and other compounds may be related to the production and stability of hemoglobin (Keven *et al*., 2003; Moreno *et al*., 2007; Bavec *et al*., 2010). In humans, beet regulates the blood pressure (Hobbs *et al*., 2012; Moreno *et al*., 2008).

Moreover, the benefits by the using of *Lactobacillus sporogenes* as a probiotic have been reported and ferments with *Lactobacillus acidophilus* demonstrated are potentiates bioavailability of iron and can result in the reduction of oxidative stress in red blood cells in models animal with mice and rats (Oda *et al*., 1994; Endres *et al*., 2009; Kaushal & Kansal, 2012).

Conclusions

Anemia recuperation with fermented beet diet may be due to one or more factors, for this reason, more studies are necessary. Our recommendations are to generate fermented combinations to evaluate the effects on anemia using other vegetables or through other kinds of bacteria.

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