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Sorghum extrusion process combined with biofortified sweet potato contributed for high iron bioavailability in *Wistar* rats

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ABSTRACT

This study aimed to evaluate the effect of sorghum and sweet potato on the bioavailability of iron, gene expression of proteins involved in iron metabolism and the plasma antioxidant capacity in animals fed with whole sorghum grains processed by dry heat or extrusion, combined or not with sweet potato flour with high content of carotenoids. Five experimental groups were tested (n = 7): dry heat sorghum flour (DS); extruded sorghum flour (ES); whole sorghum flour + sweet potato flour (DS + SP); extruded sorghum flour + sweet potato flour (ES + SP) and positive control (FS). The evaluations included: hemoglobin gain, hemoglobin regeneration efficiency, gene expression of divalente metal transporter 1 (DMT-1), duodenal citochroma B (DcytB), ferroportin, hephaestin, transferrin and ferritin and total plasma antioxidant capacity (TAC). The ES + SP group showed higher ($p < 0.05$) expression of DcytB, ferroportin and hephaestin when compared to the control group. The DS group showed high ($p < 0.05$) expression of DMT-1 and the ES showed high mRNA expression of transferrin and ferritin. The changes in the sorghum physicochemical properties from extrusion process reduced the iron and phytate content, and increased the gene expression of proteins involved in iron metabolism, improving iron bioavailability. The combination of sweet potato and sorghum flour (dry or extruded) improved the iron capture and total antioxidant capacity, probably due to the presence of β-carotene and antioxidant compounds.

1. Introduction

Iron deficiency anemia is a nutrition deficiency found in developing countries, affecting around 42% of the world population (WHO, 2015). New public health programs have been developed to control micronutrient deficiencies, such as biofortification food (Bouis et al., 2011; La Frano et al., 2014).

Sorghum (*Sorghum bicolor* L.) is the fifth cereal with the highest productivity in the world and its nutritional composition suggests that it is a source of vitamins and minerals as well as bioactive compounds (phenolic compounds and tannins) which can reduce iron bioavailability (Cardoso et al., 2015a; Petry et al., 2010; Moraes et al., 2012; Hart et al., 2015). Sorghum genotypes are an important source of iron for many people (Ashok Kumar et al., 2013) despite its low iron bioavailability. Thus, Embrapa Milho e Sorgo (Brazilian Agricultural Research Corporation) selected within a database of 100 genotypes, one with high iron, zinc, antioxidant activity, total phenolics and tannins contents (Cardoso et al., 2015a).

To minimize the effect of phytochemicals in the sorghum and enhance the iron absorption, it has been used on diets with pro-vitamin A carotenoid (Layrisse et al., 1998). Embrapa has been producing Beauregard sweet potato (*Ipomoea batatas*) with high content of carotenoids, which in association with sorghum can increase the iron bioavailability. Vitamin A can act in iron mobilization of organic tissue stocks, favoring the availability of this

Abbreviations used: ANOVA, analysis of variance; DcytB, duodenal citochroma B; DMT-1, divalente metal transporter 1; DS, dry heat sorghum flour; DS + SP, whole sorghum flour + sweet potato flour; ES, extruded sorghum flour; ES + SP, extruded sorghum flour + sweet potato flour; FER, food efficiency ratio; FS, positive control; HRE, hemoglobin regeneration efficiency; RBV HRE, biological hemoglobin regeneration efficiency; TAC, total plasma antioxidant capacity.

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mineral for hematopoiesis and hemoglobin synthesis (Semba and Bloem, 2002).

The iron homeostasis is controlled by transcriptional mechanisms that regulate gene expression of proteins involved in this mineral metabolism (Tako et al., 2013). The duodenal cytochrome b (Dcytb), divalent metal carrier protein (DMT-1), ferroportin and duodenal hephaestin are proteins that regulate iron absorption, while transferrin and ferritin are proteins synthesized in the liver and act in the transport and storage of iron, respectively (Tako et al., 2013; Grotto, 2008).

There are no studies about the iron bioavailability interference of the extrusion process combined with high carotenoid content crops. Thus, it is important to assess the effect *in vivo* of sorghum flour combinations (dry heat or extruded) with biofortified sweet potato. We aimed to evaluate the effect of the heat treatment and extrusion process of sorghum flour and its combination with high carotenoid content crop on the iron bioavailability, gene expression of proteins involved in iron metabolism, and their antioxidant effect.

2. Materials and methods

2.1. Sorghum and sweet potato

Dry or extruded whole sorghum (*Sorghum bicolor* L.) of SC 319 genotype (origin: Uganda; brown pericarp with proanthocyanidins and 3-deoxidanthocyanidins − 3DXAs) was used. The seeds were grown at Embrapa Milho e Sorgo (Nova Porteirinha, Minas Gerais, Brazil) between June and October 2011. The experimental plots were composed of two rows of 3 m long with spacing of half a meter between rows. The fertilization at planting consisted of the application of 300 kg/ha of formulated 08-28-16 (NPK). After 25 days of planting, fertilization with 50 kg/ha N was performed. Sweet potato (*Ipomoea batatas*), registered in National Register of Cultivars under the name Beauregard (number 26934) with high concentration of carotenoids were supplied by Embrapa Meio Norte (Terezina, Piauí, Brazil). The sweet potato was planted at a spacing of 1 m between furrows and 30 cm between plants. The vines were planted to between 25 and 35 cm or at least 5 gems, buried at the top of the windrow, leaving 1/3 out. The harvest took place between 120 and 150 days after growth. The productivity may range from 20 to 40 tonnes per ha, may exceed that value in cases of more fertile soil. After the harvest, the foods were stored in insulated boxes and then packed in polyethylene bags and stored at −18 ± 1 °C.

2.2. Staple food crop flours preparation

The SC 319 sorghum grains were subjected to two processing methods, dry heat/milling (DS); and extrusion/milling (ES), as described below:

DS - The whole grains were subjected to dry heat (121 °C; 25 min) in an oven with forced air circulation (Marconi®, MA 093, São Paulo, Brazil). Subsequently, the grains were ground in a mill (850 μm screen) (Marconi®, MA 090, São Paulo, Brazil).

ES - The extrusion process of sorghum flour was conducted at Embrapa Agroindústria de Alimentos Rio de Janeiro, Brazil. The whole grains previously milled (850 μm) were processed in a co-rotating twin-screw model Evolum HT 25 (Clextral, Firminy, France) (temperature between 30 and 150 °C) (Vargas-Solórzano et al., 2014). To compensate moisture differences in the samples and provide a final moisture content of 12%, distilled water was injected between the first and second feeding zones using a plunger metering pump model J-X 8/1 (AILIPU Pump Co. Ltd., China). The samples were collected over 15–20 min. Subsequently, the extrudate was ground in a mill (850 μm) (Marconi®, MA 090, São Paulo, Brazil) and stored in polyethylene bags, at −18 ± 1 °C, until analysis.

The sweet potatoes were peeled and sliced on multiprocessor (Philips Walita, Amsterdam, Netherlands) and dried in an air oven for 6 h at 60 °C (Dias et al., 2015). The sweet potato was ground in a 090 CFT mill (Marconi, São Paulo, Brazil) at 3000 rpm, sieved (600 mesh screen). All samples were stored in a freezer (−18 ± 1 °C).

2.3. Chemical composition

The determination of iron content of food and the iron content of the diet were performed according to Gomes (1996). Briefly, 1.0 g of the samples was oxidized with 10 mL of nitric acid for 8 h at room temperature. Later, the samples were heated in the digester block with exhaust to approximately 120 °C for 16 h. The iron and zinc concentrations were determined by coupled plasma atomic emission spectrometry (model Optima 3300 DV, Perkin Elmer, MA, USA), with an inducible plasma argon source. Analysis was performed under the following conditions: power of 1300 W, plasma argon flow rate of 15 L min^{-1}, auxiliary argon flow rate of 0.7 L min^{-1}, nebulizer argon flow rate of 0.5 L min^{-1}, rate of sample introduction of 1.5 mL min^{-1}. Calibration curves were used to prepare standard solutions of iron concentration, according to Cardoso et al. (2015b). The analyses were performed in triplicate.

The phytate content was quantified according to the method proposed by Latta and Eskin (1980), with modifications by Ellis and Morris (1986). For the extraction of phytates, 0.1 g in triplicate was weighed and 15 mL of HCl 2.4% were added, remaining under horizontal stirring for 12 h at 250 rpm. The extract was vacuum-filtered on büchnner funnel and purified using ion exchange column, stationary phase consisting of AG® 1-X4 Resin (Bio-Rad Laboratories, São Paulo, SP, Brazil). The column was preconditioned with NaCl 2 M and the extract obtained from the previous steps was applied to it. The inorganic phosphors were eluted with NaCl 0.05 M followed by elution of the retained phytates with NaCl 2 M. Phytate was determined colorimetrically, based on the pink coloration of Wade's reagent, formed from the reaction between ferric ion and Sulfosalicylic acid, which exhibits maximum absorbance at 500 nm. An analytical curve of phytic acid (myo-inositol hexaphosphate) (Sigma-Aldrich®, St. Louis, MO, USA, code P8810) at concentrations of 10–100 μg mL^{-1} was created using the Linear regression equation ($y = -0.0035x + 0.4818$; $R^2 = 0.9965$) to express the phytate content in milligram of phytic acid per gram of sample.

The concentration of pro-vitamin A carotenoid (α and β-carotene) in pumpkin and sweet potato was determined by Rodriguez et al. (1976). Five grams of sample were ground in 60 mL of chilled acetone for approximately 2 min and the material was vacuum filtered on a Buchner funnel using filter paper. The filtrate was transferred to a separator funnel, in which 50 mL of cooled petroleum ether were added to transfer the pigment to the acetone ether. Each fraction was washed three times with distilled water to remove all acetone. The concentration of material was performed by evaporation of the petroleum ether extract using a rotary evaporator at 35 °C. The pigments were dissolved again in a known amount of petroleum ether and stored in amber glass vials at 18 °C. For analysis, an aliquot (2 mL) of the extract stored in petroleum ether was evaporated under nitrogen flow and then recovered in the same amount of methanol and filtered through a filter unit with 0.45 μM porosity. The analyses of carotenoids were performed in triplicate by high performance liquid chromatography (HPLC) using the chromatographic conditions developed by Pinheiro-Sant'Ana et al. (1998) as follows: HPLC-DAD system (diode array detector); chromatographic column Phenomenex Gemini RP-18,

250 × 4.6 mm, 5 μm, equipped with Phenomenex ODS guard column (C18), 4 mm × 3 mm. The mobile phase was methanol: ethyl acetate: acetonitrile (80:10:10, v/v/v) at a flow rate of 2.0 mL/min.

2.4. Animals and diets

Controlled experimental tests were used and the bioavailability of iron was evaluated by the hemoglobin depletion/repletion method (AOAC, 1974 – Method 974.31). At 21 days of age, 35 male rats (*Ratus norvegicus albinus* Wistar) from the Central Animal Facility of the Center for Life Sciences and Health at Federal University of Viçosa, Minas Gerais, Brazil, were placed in individual temperature-controlled (22 ± 2 °C) cages, with a photoperiod of 12 h. The experimental diets were based on the standard AIN-93G diet (Reeves et al., 1993) (Table 1).

Animals initially received a depletion diet containing Fe-free mineral mixture to reduce hemoglobin concentrations and deionized water *ad libitum*, for 21 days. Animals were then divided into five groups (n = 7) such that the hemoglobin concentration was not statistically different among groups: (1) control (containing ferrous sulfate - FS); (2) dry heat sorghum flour (DS); (3) extruded sorghum flour (ES); (4) dry heat sorghum flour + sweet potato flour (DS + SP); (5) extruded sorghum flour + sweet potato flour (ES + SP). The repletion diet was pair fed to control food and iron intake, and deionized water was offered *ad libitum*, for 14 days.

During the repletion phase the sorghum and sweet potato flour were used as source of iron, and the ferrous sulfate was used as positive control. All treatments offered 12 mg of iron per kg of diet (Silva et al., 2016) (Table 1). The weight gain and food consumption weekly for the calculation of food efficiency ratio was (FER = [weight gain (g)/food intake (g)] x 100). At the end of the repletion phase, the hemoglobin concentration was performed for calculating the hemoglobin gain.

On the 36th day, after 12 h fasting, the animals were anesthetized with isoflurane (Isoforine, Cristália, Itapira, Brazil) and were euthanized by cardiac puncture. Blood and fragments of the liver and duodenum were collected. All experimental procedures with animals were performed in accordance with the ethical principles for animal experimentation and the study approved by the Ethics Committee of the Federal University of Viçosa, Brazil (Protocol 09/2014).

2.5. Blood tests

Serum hemoglobin was measured using a colorimetric kit (Bioclin®, Brazil) for *in vitro* diagnosis. A volume of 20 μL of blood was pipetted and mixed with 5 mL of Drabkin's solution color regent (containing potassium cyanide, and hydrogen cyanide). The absorbance reading was performed in UV–Visible Multiskan (Thermo Scientific, Massachusetts, MA, USA) at 540 nm wavelength.

2.6. Iron bioavailability

The iron bioavailability was calculated according to Hernández et al. (2003). The hemoglobin (Hb) regeneration efficiency (HRE %) was calculated by the equation: HRE % = (mg Fe final Hb - mg Fe initial Hb)/mg Fe consumed * 100. The iron in hemoglobin content was estimated by: [Body weight (g) * Hb (g/L) * 0.335 * 6.7]/1000. This variable was calculated assuming the total blood volume equals to 6.7% of the rat's body weight, and the body iron in hemoglobin content as being 0.335 g/L.

2.7. Extraction of mRNA from liver and duodenal tissue and cDNA synthesis

The tissues were macerated in liquid nitrogen in RNAse free conditions and samples were aliquoted for total RNA extraction. Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) using the manufacturer's recommendations. 2 μL of mRNA extracted was used to synthesize the cDNA using M-MLV reverse transcription kit (Invitrogen Corp., Grand Island, New York), according to the manufacturer's protocol.

Table 1
Food and nutritional composition of diet AIN 93-G.

Ingredients (g Kg^{-1})	Depletion Phase	FS	DS	ES	DS + SP	ES + SP
Ferrous sulfate	–	0.06	–	–	–	–
Dry heat sorghum	–	–	274.00	–	194.20	–
Extruded sorghum	–	–	–	374.00	–	240.00
Sweet potato flour		–	–	–	194.20	240.00
Albumin	200.00	200.00	164.10	148.70	168.10	159.10
Dextrinized starch	132.00	132.00	132.00	132.00	132.00	132.00
Sucrose	100.00	100.00	100.00	100.00	100.00	100.00
Soybean oil	70.00	70.00	65.00	62.00	63.40	61.40
Microcrystalline cellulose	50.00	73.30	27.40	11.00	14.00	–
Mineral mix without iron	35.00	35.00	35.00	35.00	35.00	35.00
Vitamin mix	10.00	10.00	10.00	10.00	10.00	10.00
L-cystine	3.00	3.00	3.00	3.00	3.00	3.00
Choline bitartrate	2.50	2.50	2.50	2.50	2.50	2.50
Corn starch	400.00	365.00	189.00	124.00	86.00	19.00
Nutritional composition						
Total calories (Kcal)	3847.88	5447.96	4017.80	3864.89	5447.72	5364,67
Caloric density (kcal g^{-1})	4.15	5.65	4.20	4.17	5.65	5.58
Iron (mg Kg^{-1})	0.00	18.53	25.84	23.87	20.68	22.17
Molar ratio phytate/iron*	–	–	53.73 ± 0.95[b]	58.59 ± 0.95[b]	31.39 ± 0.76[a]	30.02 ± 0.58[a]

Ferrous sulfate (FS); dry heat sorghum flour (DS); extruded sorghum flour (ES) and sweet potato flour (SP). Adapted by Reeves, Nielsen and Fahey [21]. The contents of protein, lipid and total dietary fiber were discounted from the DS: protein = 2.84 g.100 g^{-1}, lipid = 0.5 g.100 g^{-1}, total dietary fiber = 4.58 g.100 g^{-1}. ES: protein = 4.06 g.100 g^{-1}, lipid = 0.76 g.100 g^{-1}, total dietary fiber = 6.25 g.100 g^{-1}. DS + SP: protein = 2.52 g.100 g^{-1}, lipid = 0.66 g.100 g^{-1}, total dietary fiber = 5.93 g.100 g^{-1}. ES + SP: protein = 3.24 g.100 g^{-1}, lipid = 0.86 g.100 g^{-1}, total dietary fiber = 7.32 g.100 g^{-1}. Means followed by different lower case in line differ by Duncan teste ($p < 0.05$).
*Iron (3.30 mg/100 g) and phytate (100 mg/100 g) values used for molar ratio calculation were based on Dias et al. (2015).

2.8. Determination of gene expression of proteins involved in iron metabolism by reverse transcriptase polymerase chain reaction (RT-qPCR)

Expression of the mRNA levels of proteins involved in iron metabolism in the duodenal mucosa and in the liver were analyzed by RT-qPCR. The SYBR green PCR master mix from Applied Biosystems (Foster City, CA, USA) was used and analyses were performed on the StepOne™ Real-Time PCR System (Thermo Fisher Scientific) using the measurement system by SYBR-Green Fluorescence and Primer Express software (Applied Biosystems, Foster City, CA, USA). The PCR involved an initial denaturation cycle of 95 °C (10 min) and then 40 cycles with 1 min denaturation (94 °C), 1 min annealing (56 °C) and 2 min elongation (72 °C), followed by a standard dissociation curve. Sense and antisense primer sequences (Choma Biotechnologies, Rio de Janeiro, Brazil) has been previously evaluated for dimer formation and were used (Sonnweber et al., 2012) to amplify protein divalent metal carrier (DMT-1), duodenal cytochrome b (DcytB), ferroportin and hephaestin from duodenum, and proteins ferritin and transferrin from liver. The relative expression levels of mRNA were normalized by the endogenous control glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Table 2). All steps were performed using open conditions with RNase.

2.9. Total plasma antioxidant capacity

Total antioxidant capacity (TAC) of the animals was evaluated by colorimetric test with the Sigma kit (Sigma-Aldrich®). The result was expressed in mM equivalent trolox.

For the procedure, the lyophilized trolox standard was reconstituted by the addition of 2.67 mL of 1 × assay buffer. For the preparation of ABTS substrate solution, an ABTS tablet and a phosphate citrate buffer tablet was added to 100 mL of ultrapure water. Subsequently, 37.5 μL of hydrogen peroxide solution 3% were added to 15 mL of ABTS substrate solution. The assay buffer was diluted 10 times in ultrapure water. The lyophilized myoglobin was reconstituted in 285 μL of ultrapure water, and before use it was diluted 100 times in 1 × buffer (myoglobin working solution). Afterwards, 190 μL of assay buffer 1 × was added to microfuge tubes containing 10 μL plasma. Initially, 10 μL of trolox standards was pipetted in the center of the well Elisa plate in duplicate with increasing concentrations.

Aliquots of 10 μL of plasma was added to the remaining wells identified for the samples. To all the wells, it was added 20 μL of the myoglobin working solution and 150 μL of ABTS substrate solution with hydrogen peroxide. The plate was covered with adhesive and incubated at room temperature for 5 min. After incubation, 100 μL of stop solution previously heated was added. The absorbance reading was performed at 405 nm in a spectrophotometer (Thermo Scientific, Ratastie, Finland). For the calculation, the straight line equation was used ($y = -1{,}3565x + 0.6823$) obtained by the construction of the calibration curve and the values expressed in mM.

Table 2
Sequence of primers used in the RT-PCR analysis.

Genes	Oligonucleotide (5′-3′)	
	Forward	Reverse
GAPDH	AGGTTGTCTCCTGTCACTTC	CTGTTGCTGTAGCCATATTC
DMT-1	CTGATTTACAGTCTGGAGCAG	CACTTCAGCAAGGTGCAA
DcytB	TGCAGACGCAGAGTTAAGCA	CCGTGAAGTATACCGGCTCC
Ferroportin	TTCCGCACTTTTCGAGATGG	TACAGTCGAAGCCCAGGACCGT
Hephaestin	GGCACAGTTACAGGGCAGAT	AGTAACGTGGCAGTGCATCA
Ferritin	CAGCCGCCTTACAAGTCTCT	ATGGAGCTAACCGCGAAGAC
Transferrin	AGCTGCCACCTGAGAACATC	CGCACGCCCTTTATTCATGG

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH); divalent metal transporter-1 Protein (DMT-1) and duodenal cytochrome B (DcytB).

2.10. Statistical analysis

The sorghum flours were acquired from different batches and processed on three distinct days, characterizing three repetitions. The results were analyzed by ANOVA. For significant "F-value", post hoc Duncan test was used to compare the averages of all experimental groups. The mean dispersion was expressed as standard deviation. Statistical analyses were carried out using SPSS statistics version 2.0 software considering a 5% significance level.

3. Results and discussion

3.1. Effect of heat and extruded treatment on the phytate and iron concentration in sorghum flour

The iron and phytate contents was higher ($p < 0.05$) in dry heat sorghum flour than in the extruded sorghum flour (Fig. 1 A). The lower phytate contents in extruded sorghum flours can be attributed mainly to the action of phytases in the grain with can be active at temperature around 55 °C (Albarracín et al., 2015). The lower iron concentration in extruded sorghum flours can be explained by water incorporation into the food matrix during the extrusion process. In addition, factors such as temperature and water addition can disrupt matrices by altering iron stability or forming insoluble complexes that decrease their bioavailability.

3.2. Effect of the whole and extruded sorghum flour intake and their combinations with sweet potato flour on iron bioavailability

Food intake was similar ($p > 0.05$) in all groups, except in treated with extruded sorghum flour combined with sweet potato flour. Despite this result, the weight gain in this group was similar ($p > 0.05$) to the others. The test groups, except the one treated with dry heat sorghum flour, showed hemoglobin gain, hemoglobin regeneration efficiency (HRE) and biological hemoglobin regeneration efficiency (RBV HRE) similar ($p > 0.05$) to the control group (Table 3). The high iron bioavailability showed in this groups can be related with the extrusion process and biofortified sweet potato intake. The extrusion process resulted in a lower phytate concentration (Fig. 1) in sorghum flour which is an inhibitory factor of iron

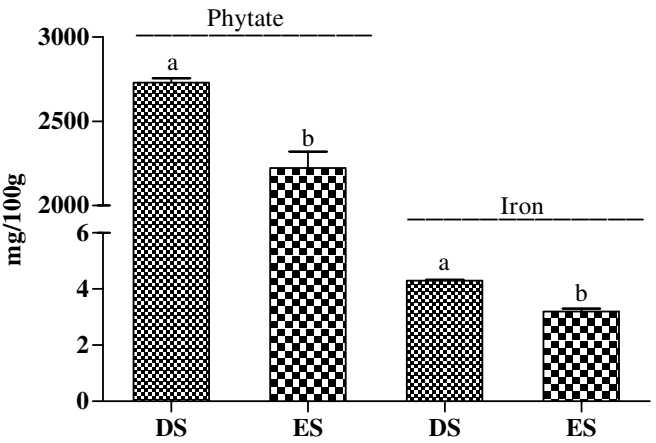

Fig. 1. Content from phytate and iron. Dry heat sorghum flour (DS) and extruded sorghum flour (ES). Different letters indicate statistical difference at 5% probability by ANOVA.

Table 3
Food intake, body weight gain, total intake of iron, hemoglobin concentration and indices for assessing iron bioavailability (n = 7).

	FS	DS	ES	DS + SP	ES + SP
Food intake (g)	200.12 ± 8.09[a]	198.75 ± 13.72[a]	207.15 ± 5.49[a]	189.37 ± 2.27[ab]	172.91 ± 31.29[b]
Body weight gain (g)	44.04 ± 12.75[a]	46.12 ± 15.73[a]	47.95 ± 10.10[a]	45.01 ± 15.74[a]	42.56 ± 13.15[a]
Iron intake (mg)	3,71 ± 0.10[b]	5,13 ± 0.16[a]	4,94 ± 0.07[a]	3,92 ± 0.24[b]	3,83 ± 0.32[b]
Initial hemoglobina (g dL^{-1})	6.18 ± 1.35[a]	6.30 ± 1.30[a]	6.20 ± 1.34[a]	6.27 ± 1.36[a]	6.22 ± 1.37[a]
Final hemoglobin (g dL^{-1})	10.21 ± 1.89[a]	8.10 ± 1.53[b]	10.09 ± 1.39[a]	10.26 ± 1.46[a]	9.48 ± 1.53[a]
Hemoglobin gain (g dL^{-1})	4.03 ± 1.07[a]	1.80 ± 1.26[b]	3.89 ± 0.83[a]	3.99 ± 1.65[a]	3.20 ± 1.08[a]
HRE%	65.04 ± 16.59[a]	27.47 ± 12.38[b]	52.32 ± 9.18[a]	62.73 ± 21.61[a]	52.40 ± 9.53[a]
RBV-HRE	1.00[a]	0.42 ± 0.19[b]	0.80 ± 0.14[a]	0.96 ± 0.33[a]	0.80 ± 0.15[a]

Data presented as mean ± standard deviation. Ferrous sulfate (FS); dry heat sorghum flour (DS); extruded sorghum flour (ES) and sweet potato flour (SP). Hemoglobin maintenance efficiency (HRE); relative biological value of HRE (RBV-HRE). Means followed by different lower case in line differ by Duncan test ($p < 0.05$).

absorption (Petry et al., 2010). Additionally, the DS group combined with sweet potato presented similar iron bioavailability to the control. This result can be attributed to biofortified sweet potato (127.11 mg/100 g of β-carotene) (Dias et al., 2015), which has pro-vitamin A carotenoid, increasing the iron absorption (Semba and Bloem, 2002). The carotenoids bind with iron forming soluble complex, which reduce the negative effect of tannin, phytate and phenolic compounds on iron absorption (Layrisse et al., 1998; Petry et al., 2010).

The diets containing sweet potato showed lower phytate:iron molar ratio then the diets containing only sorghum flour, since the sweet potato presents high iron concentration (Dias et al., 2015). Studies in humans have shown that the molar ratio of phytate: iron from 4 to 30 could significantly inhibit iron absorption (Petry et al., 2013). The combination of sorghum flour with high pro-vitamin A carotenoid content crops increased the iron absorption, even in diets with a high molar ratio of phytate: iron.

3.3. Gene expression of proteins involved in iron metabolism

It was observed, through the evaluation of transcriptional mechanisms which regulates iron metabolism, that the group fed with sweet potato (dry or extruded sorghum flour) showed higher ($p < 0.05$) gene expression of ferroportin (Fig. 2a). This result can be attributed to carotenoids presents in sweet potato which is converted into retinoid acid in the body. This acid can induce the gene expression of ferroportin (Citeli et al., 2012).

The groups treated with ES and ES + SP had higher ($p < 0.05$) gene expression of DcytB (261 and 286 times, respectively) (Fig. 2d) and transferrin (10.8 times and 7.8 times, respectively) (Fig. 3b)

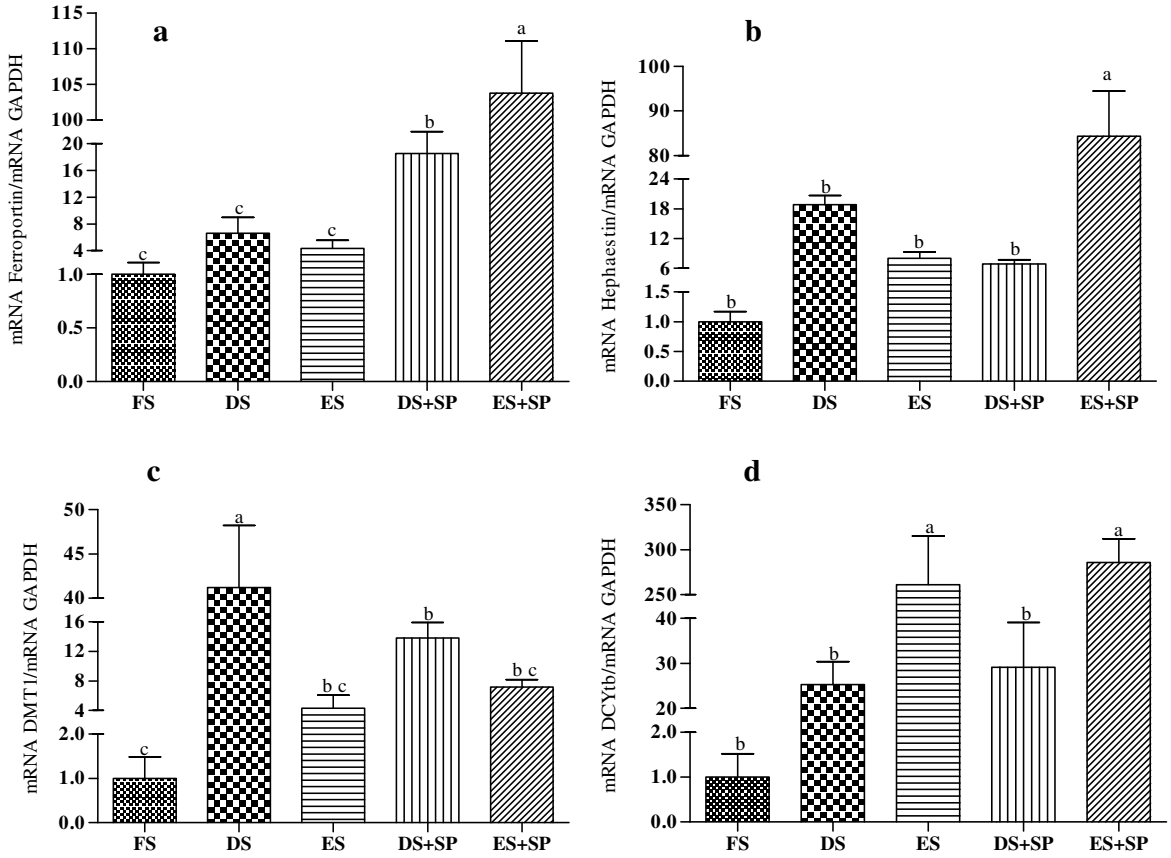

Fig. 2. Effect of the dry heat and extruded sorghum flour intake and their associated with sweet potato flour and ferrous sulfate on proteins gene expression in the duodenal tissue. Ferrous sulfate (FS); dry heat sorghum flour (DS); extruded sorghum flour (ES); sweet potato flour (SP). Analysis by RT-PCR. (**a**) ferroportin (**b**) Hephaestin (**c**) DMT-1 (**d**) Dcytb. Different letters indicate statistical difference at 5% probability by the Duncan test.

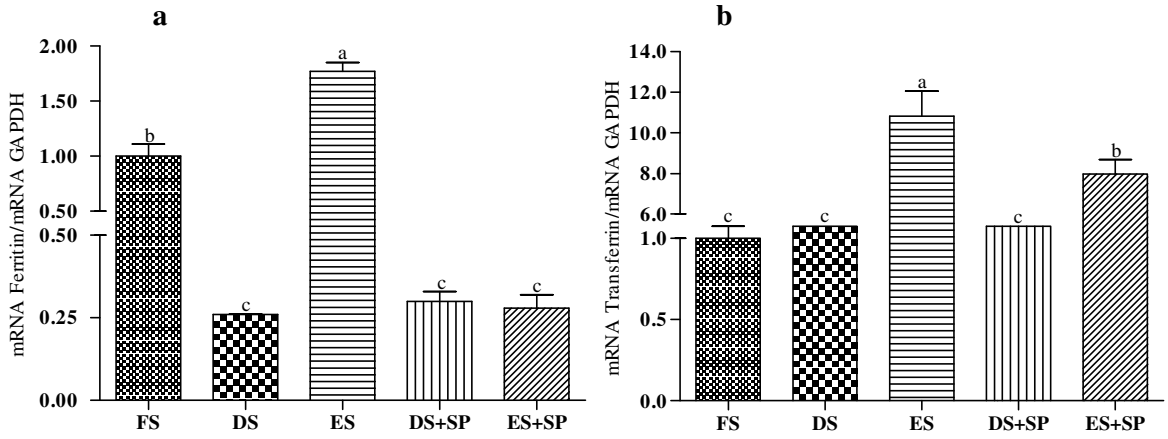

Fig. 3. Effect of the dry heat and extruded sorghum flour intake and their associated with sweet potato flour and ferrous sulfate on gene expression of ferritin and transferrin in the liver. Analysis by RT-PCR. (**a**) Ferritin (**b**) Transferrin. ferrous sulfate (FS); dry heat sorghum flour (DS); extruded sorghum flour (ES); sweet potato flour (SP). Different letters indicate statistical difference at 5% probability by the Duncan test.

compared to the control group. Also, the group treated with ES + SP showed higher expression of hephaestin than the other groups (Fig. 2b) and the group treated with ES had higher ($p < 0.05$) gene expression of ferritin compared to the others groups (Fig. 3a).

The group treated with dry heat sorghum flour had similar iron intake to other groups, but smaller hemoglobin gain and consequently lower efficiency of hemoglobin regeneration. This result indicates a lesser quantity of bioavailable iron in this diet. In addition, this group had high gene expression of DMT-1 (Fig. 2c) compared to the others, suggesting the compensatory mechanism due to the deficient iron status and a lower amount of bioavailable iron. Furthermore, this group showed lower ferritin expression than the control that occurs because the animals had a lesser supply of bioavailable iron.

3.4. Effect of the test food intake on the total plasma antioxidant capacity

All test groups had higher antioxidant capacity than the control group ($p < 0.05$). Thus, the whole sorghum flours (dry heated or extruded) and their combinations with biofortified sweet potato flour with carotenoids improved the total antioxidant capacity of plasma.

Regarding the TAC of the plasma of the animals, the test groups showed higher TAC than the control group. Corroborating this result, Moraes et al. (2012) also reported an increase in TAC in animals fed with whole sorghum flour. This is because sorghum has high content of phenolic compounds, such as 3- deoxyanthocyanins, which increases the antioxidant capacity of this cereal, regardless of processing (Cardoso et al., 2015a). Furthermore, this sweet potato flour contains high concentration of carotenoids, which sequester reactive oxygen species inhibiting oxidative damage (Farrar et al., 2008; Giese et al., 2015).

4. Conclusion

The changes in the sorghum physicochemical properties from extrusion process reduced the iron and phytate content, and increased the gene expression of proteins involved in iron metabolism, improving iron bioavailability. In addition, the combination of sweet potato and sorghum flour (dry or extruded) improved the iron capture and TAC, probably due to the presence of β-carotene and antioxidant compounds.

Compliance with ethical standards

All procedures performed in this study involving animals were in accordance with the ethical standards of the Federal University of Viçosa.

Conflict of interest

The authors declare that they have no conflict of interest.

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