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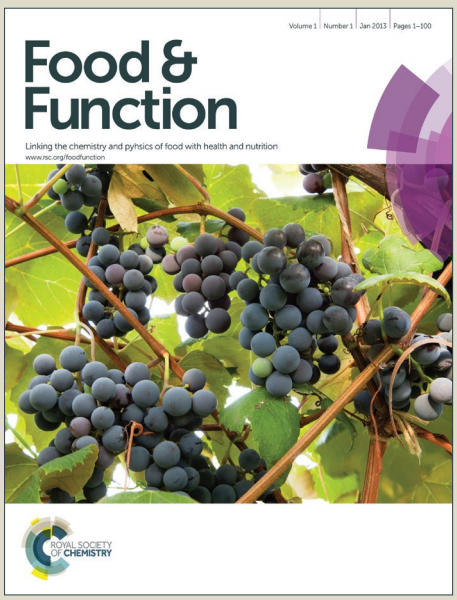

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Inclusion of ancient Latin-American crops to bread formulation improves intestinal iron absorption and modulates inflammatory markers

José Moisés Laparra, and Monika Haros

[1] Instituto de Agroquímica y Tecnología de Alimentos (IATA). Consejo Superior de Investigaciones Científicas (CSIC). Av. Agustín Escardino 7, Parque Científico, 46980 Paterna-Valencia, Spain

***Corresponding author:** **José Moisés Laparra**

Avda. Agustín Escardino 7

46980 Paterna

Telephone: +343 900 022

Fax: +34 963 636 301

E-mail: j.moises.laparra@uv.es

Abstract

This study compares iron (Fe) absorption in Fe-deficient animals from bread formulations prepared by substitution of white wheat flour (WB) by whole wheat flour (WWB), amaranth flour (*Amaranthus hypochondriacus*, 25%) (AB) and quinoa flour (*Chenopodium quinoa*, 25%) (QB), or chia flour (*Salvia hispanica L*, 5%) (ChB). Hematological parameters of Fe homeostasis, plasmatic active hepcidin peptide production (LC coupled to Ms/Ms), and liver TfR-2 and IL-6 expression (RT-qPCR) were determined. The different bread formulations increased Fe content between 14% and 83% relative to white bread. Only animals fed with WWB, AB and ChB increased significantly haemoglobin concentrations. Feeding the different bread formulations did not increase hepcidin levels, but down-regulated TfR2 (except WWB) and IL-6 (except QB) expression levels. Only AB and ChB had significant influence favoring on Fe bioavailability at the investigated level of substitution. The potential contribution of these flours would not differ considerably from that of WWF.

Keywords: Iron absorption, bread, amaranth, quinoa, chia, phytates.

1. Introduction

Iron (Fe) deficiency is a major cause of detrimental health effects in children and women of reproductive age because, among other, impairing mental and psychomotor development, increasing both morbidity and mortality of mother and child and decreasing work performance.[1] This worldwide health problem shows that Fe-deficiency continues to be a problem and points to the need of developing preventive nutritional intervention strategies in relation to this micronutrient. A desirable, easy and low cost strategy to prevent Fe-deficiency is, when possible, dietary diversification to promote the consumption of available mineral-rich foods.

Cereals constitute a good source of iron and bread, as staple food with high consumption frequency, can serve as a good vehicle to combine those with natural ingredients improving its nutritional value. Nutritional and chemical composition analyses indicate that the pseudocereals, among other, amaranth (*Amaranthus cruentus*) and quinoa (*Chenopodium quinoa*), as well as oliseeds such as chia (*Salvia hispanica L*) can represent a healthier alternative to frequently used wheat grain in bread formulations. For example, there have reported 2.8- and 1.7-fold higher Fe contents in amaranth and quinoa seeds than in wheat grains, respectively.[2] Whole amaranth flour (WAF) used in bread formulation increases the Fe content in bread samples up to 2.3-fold (43.8 μg/g) in comparison to white bread. [3] There have been quantified 29.5 μg/g Fe concentrations in quinoa grains that are higher than those values found in rice (13.2 μg/g) and finger millet (21.3 μg/g). [4] In this line, the oilseed chia is also a good source of Fe (164.0 mg/g) and once processed its flour can provide up to 20.4 mg/g. [5] Additionally, the breads containing pseudocereals have also significant higher levels of protein, fat and fibre than white bread, even at low levels of substitution. Moreover, the

attributes of these breads conform to the expert's nutritional recommendations for the gluten-free diet and gluten-free foods.

Only scarce *in vivo* studies have been performed about to what extent the pseudocereals (amaranth or quinoa) impact the nutritional iron status. [6,7] Currently, it is assumed the higher nutritional value of pseudocereals and the oilseed chia; however, there is a lack of data supporting their positive influence improving the nutritional Fe status of iron-deficient experimental models. Pseudocereals as well as conventional cereals carry a significant amount of phytic acid, a potent inhibitor of Fe bioavailability. [8,9] However, the absorption of dietary Fe is also regulated by physiological factors, among other, bioactive hepcidin peptide. [10-12] The latter is a key regulator of Fe metabolism associated to acute phase inflammatory processes within the gut-liver axis. [11,13] However, understand the interaction of phytic acid and hepcidin on Fe absorption is not straightforward. For example, there have been reported similar percentages of Fe absorption in girls and boys after consumption of two different diets with a 2.6-fold unfavourable ratio phytic acid/Fe. [6] Phytic acid has shown to exhibit protective action and attenuate inflammation in several different rodent models of Parkinson's disease [14] and cancer [15]. These diseases constitute pathological conditions where hepcidin plays an important role favouring metal dyshomeostasis and inflammation. [16,17]

Although there is a number of differences concerning Fe absorption in rats compared with humans limiting their predictive value in relation to human responses, [18] it is worth to study and understand the phytic acid-Fe interactions in foods with high nutritional potential. Herein, it was used a nutritionally-induced iron deficient animal model that exhibits comparable responses of regulatory factors of systemic Fe homeostasis that involve liver transferrin receptor-2 and hepcidin. [11,12,19]

The present study evaluated the absorption of Fe contained in several different bread formulations containing amatranth, quinoa or chia. The different bread formulations were also compared according to their influence on hepatic pro-inflammatory hepcidin peptide secretion that exerts a critical control on intestinal Fe absorption and hepatic metabolism.

2. Material and methods

2.1 Breadmaking. Commercial Spanish wheat flour and whole wheat flour were purchased from the local market (La Meta, S.A., Spain) as well as quinoa kernels (*Chenopodium quinoa*) (Ecobasic – Bio, S.L., Spain), amaranth kernels (*Amaranthus hypochondriacus*) (Corporación Proteína Americana, SCRL, Tehuacán Puebla, Mexico) and chia seeds (*Salvia hispanica*) (Primaria Raw Matrials, Valencia-Spain). Compressed yeast (*Saccharomyces cerevisiae*, Levamax, Spain) was used as a starter in identical breadmaking processes according to previously established processes. [3,20,21]

2.2 Determination of phytates. InsP_6 present in flours and the remaining InsP_6 in bread were purified by ion-exchange chromatography and measured by the HPLC method described by Sanz-Penella et al. [3] Identification of phytates was achieved by comparison with standards of phytic acid di-potassium salt (Sigma-Aldrich, St. Louis, MO). Samples were analyzed in triplicate.

2.3 Determination of iron. The total Fe concentration in bread samples was determined using a flame atomic absorption spectrometer at the Servei Central de Suport a la Investigació Experimental from the Universitat de València. Previously, samples were placed in a Teflon perfluoroalkoxy (PFA) vessel and treated with 1 ml HNO_3 (14 M, Merck) and 1 ml of H_2O_2 (30 % v/v, Panreac Quimica, Spain). The Teflon PFA vessel was irradiated at 800 W (15 min at 180ºC) in a microwave

accelerated reaction system (MARS) from CEM (Vertex, Spain). At the end of the digestion program, the digest was placed in a tube and made up to volume with 0.6 M HCl (Merck). Samples were analyzed in triplicate (n=3).

2.4 Animals. Forty-two female Wistar albino rats, aged 3 weeks with an average weight of 61.4 ± 5.6 g were obtained from the University of Valencia Animal Service. Animal experiments were carried out in strict accordance with the recommendations included in the Guide for the Care and Use of Laboratory Animals of University of Valencia (SCSIE, University of Valencia, Spain) and the protocol was approved by its Ethic Committee (A1351244049254).

2.5 Experimental design. Animals were randomly distributed into eight different groups (n=7 per group), (1) a control group receiving a standard AIN-93G diet, and seven iron-deficient groups receiving a AIN-76A diet (Harlan) for 15 days that were subjected to different treatments: (2) iron-deficient group; (3) administered with $FeCl_3$ (2.5 mg), or administered with different bread formulations to provide a similar amount of the micronutrient, (4) white bread; (5) amaranth flour-containing bread; and (6) chia flour-containing bread; (7) quinoa flour-containing bread; and (6) whole wheat flour-containing bread. A 0.1 g aliquots of the different samples were administered intragastrically (gavage) three times per day during two consecutive days. The rats were maintained in an environment of controlled temperature (21–23 ºC), humidity (55%) and light (12 h)–dark (12 h) cycle, with ad libitum food and mineral-free water available. Records of weight and food intake were collected daily. After treatment, rats were anaesthetised (isofluran) and sacrificed by exsanguination. Whole blood samples were preserved in EDTA-treated tubes for haematological analyses and the rest of the blood was used for hepcidin peptide quantification. Sections (±100 mg) of the liver

were immersed in RNA later buffer (Qiagen, CA, USA) and snap-frozen in liquid nitrogen for gene expression analyses.

2.6 Hemoglobin (Hb) measurement. Hb concentrations were measured photometrically using cyanmethemoglobin standard solution according to the manufacturer's instructions (Sigma-Aldrich). This method is based on the oxidation of Hb and its derivatives (except sulfhemoglobin) to methemoglobin in the presence of potassium ferricyanide to form cyanmethemoglobin. The absorbance, measured at 540 nm, is proportional to the total Hb concentration.

2.7 Hematological parameters. The number of erythrocytes was calculated by using a Neubauer improved cell counting chamber and hematocrit was estimated by centrifugation of whole blood in microcapillar tubes. The mean corpuscular volume (MCV) was calculated using the following equation: (hematocrit x 10)/number of erythrocytes (10^6 per mm^3 blood), and mean corpuscular Hb (MCH) (%) as: (hemoglobin (g/dL) x 100)/hematocrit. The globular sedimentation speed (VSG) was determined according to Westergren's method as proposed by the International Council for Standardization in Hematology (ICSH).

2.8 Reverse transcription and real-time PCR analyses. [12] Total mRNA was extracted from liver tissue samples using an RNeasy mini kit (Qiagen, USA) following the protocol provided by the manufacturer. One microgram of total mRNA was converted to double-stranded cDNA using AMV Reverse Transcriptase (Promega, USA). PCR was performed with primers designed for the following *Rattus norvegicus* genes: TfR2 (forward: 5'- GGC AGA GTG GTC GCT GGG TG -3'; reverse: 5'- GGC CAG AGC TCG GCA GTG TG -3'); IL-6 (forward: 5′-TCT CGA GCC CAC CAG GAA C -3′; reverse: 5′-AGG GAA GGC AGT GGC TGT CA -3′) and β-actin (forward 5'- CTC TTC CAG CCT TCC TTC CT-3'; reverse 5'- TAG AGC CAC CAA TCC

ACA CA-3'), the latter was used as a housekeeping gene. The PCR mix (20 μL reaction volume) consisted of 7.5 μL SYBR Green I master mix, 1.3 μmol/L primers, and 2.5 μL cDNA. PCR reactions were performed in triplicate in a LightCycler® 480 (Roche) with the following program: 1 cycle at 95 ºC for 5 min, 35 cycles at 60 ºC for 20 s and 72 ºC for 45 s. The relative mRNA expression of the tested genes was normalized using β-actin as housekeeping gene applying the $2^{-\Delta Cp}$.

2.9 Quantification of hepcidin in plasma (LC-Ms/Ms). All sample preparation steps were performed at room temperature as previously described. [11] Aliquots (50 μL) of plasma were pipetted into 200 μL cone tubes and a 100 μL aliquot of acetonitrile (Burdick and Jackson, Muskegon, MI) was added to each tube and mixed by pipetting. The samples were then centrifuged at 3,000 x*g* for 10 minutes at 4°C (Jouan, Winchester, VA). After centrifugation, the protein precipitation supernatant (100 μL) was mixed with 0.02% (v/v) aqueous acetic acid and injected on an Agilent HPLC system connected on line to an Esquire-LC electrospray system equipped with a quadrupole ion trap mass spectrometer (Bruker Daltonics, Billerica, MA). The HPLC system was equipped with a quaternary pump, an in-line degasser, an automatic injector, and a variable wavelength absorbance detector set at 214 nm (1100 Series, Agilent Technologies, Waldbronn, Germany). The column used in these analyses was a BioBasic C18 5 μm 4.6x250 mm (Thermo, Waltham, MA, USA). The mobile phases consisted of trifluoroacetic acid/isopropanol/water (0.125/1/500) (A) and trifluoroacetic acid/isopropanol/water/methanol/acetonitrile (0.125/1/50/350/100) (B). Aliquots (50 μL) of the precipitation supernatants were injected in each cycle and the analysis was performed with the following gradient (minutes, %B): 0, 5; 30, 90; 33, 100; 35, 0; 40, 90; 45, 5.

The *m*/*z* spectral data were processed and transformed to spectra representing mass values using the program Data Analysis version 3.0 (Bruker Daltonics). BioTools version 2.1 (Bruker Daltonics) software was used to process the Ms/Ms spectra and to perform peptide sequencing. Two independent samples from each animal were analyzed.

2.10 Statistical analysis. Statistical analyses were performed using SPSS v.15 software (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) and the Tukey *post hoc* test were applied. Variance analysis by one-way method was used to compare the influence of feeding different bread formulations in the iron-deficient groups of animals. Individual means were tested using pair-wise comparison with Tukey's multiple comparison tests when effects were significant. Statistical significance was established at $P<0.05$ for all comparisons. Data are expressed as mean ± standard deviation.

3. Results

3.1 Micronutrient contribution of bread formulations and influence on hematological parameters. Total iron (Fe) and phytate (InsP_6) content in different bread formulations are shown in **Table 1**. Substitution of wheat flour by the different types of flour increased Fe content between 14% and 83% relative to control (white bread, WB). The concentration of InsP_6 also increased following the gradation: AB > WWB > QB > ChB. However, concentration of InsP_6 was below the quantification limit in WB samples. When considering the total Fe contents and InsP_6 concentration in the different bread formulations there can be calculated values for the ratio InsP6/Fe that are ranged between 1.6 and 4.7. These values are markedly higher than the critical value (>1) established as inhibitory for Fe uptake. [22] Thus, there can be expected low

bioavailability values for the micronutrient from bread formulations with the different flours from the pseudocereals (amaranth and quinoa) or chia used.

Animals fed with the iron-deficient diet alone exhibited significant lower hemoglobin (Hb) concentrations in relation to control animals (**Table 2**). In agreement with previous studies there was detected no significant alterations in the hematocrit [12] that is estimated to appear feeding the iron-deficient diet for longer periods (>20 days). [23] Nevertheless, there was a significant decrease in the mean corpuscular hemoglobin concentration (MCH) ($P < 0.05$) in animals receiving the Fe-deficient diet. Animals administered with the supplement of Fe alone showed significant increases in Hb (46%) and MCH (42%). Whereas iron deficient (ID) animals fed with AB and ChB also showed increased Hb concentration those fed WWB as well as WB or QB did not increased their Hb values indicating the lower bioavailability of the micronutrient from these samples. Only those animals fed ChB showed significant increases in MCH values; however, all other groups of treatment showed increasing trends ($p>0.05$) for MCH values. In ID animals, MCV values were increased in those groups administered with AB, ChB and QB. There were not detected significant ($P>0.05$) differences neither in body weight nor number of erythrocytes among the different experimental animal groups (*data not shown*).

3.2 Influence of bread formulations on plasma active hepcidin peptide and hepatic biomarkers. The active hepcidin peptide was identified by sequencing its amino acid backbone by using a previously optimized protocol for analysis in rat serum [11] (**Figure 1**). The consumption of the ID diet did not cause significant ($P>0.05$) alterations in bioactive hepcidin peptide production relative to control animals. Notably, animals administered with the supplement of Fe alone exhibited significantly increased (1.4-fold) circulating hepcidin concentrations. Animals administered with the different

bread formulations did not provoke significant elevations in the circulating concentration of hepcidin demonstrating the physiological liver inflammatory response associated to intrahepatic iron accumulation. [11] Serum hepcidin displays an inverse relationship with Fe intestinal absorption either from foods or dietary supplements. [10]

Changes in hepatic transcripts (mRNA) of transferrin receptor (Tfr)-2 and interleukin (IL)-6 in the different groups of treatment are shown in **Figure 2**. The administration of all bread formulations, except WWB, down-regulated the expression levels of Tfr2 that relies information on plasmatic concentrations of the micronutrient. [24] Overall, animals from the different groups of treatment showed down-regulated expression levels of IL-6 that is a key inducer of hepatic acute-phase protein production and plays an important role in haematopoiesis, inflammation and immune regulation. [13]

4. Discussion

Pseudocereals and oilseeds are increasingly used to obtain flours and supplement bread formulations in order to improve their nutritional value. The quantification of total Fe in the analyzed bread formulations is in good agreement with previous studies supporting the nutritional profiles of quinoa (*Chenopodium quinoa*), amaranth (*Amaranthus Caudatus*) [21,25,26,27], and chia as rich sources of the micronutrient. [20] In the scientific literature scarce *in vivo* studies evaluated the impact of amaranth grains [3,7] or quinoa on the nutritional iron status. [6] In accordance with these previous studies the data reported in this study point out significant differences in Fe bioavailability from the different bread formulations. These differences are supported by the different ratios (values from 0.76 to 1.1) that can be calculated between Hb concentrations in animals fed with bread formulations and those administered with a reference dose of inorganic Fe. Previous data from experimental models also reported a low or negligible influence

of amaranth improving the bioavailability of the micronutrient to growing Sprague-Dawley weaning male rats. [28,29] Intervention studies in human subjects without ID that were fed with cereals fortified with 2.5 mg Fe as $FeSO_4$ it was also demonstrated low bioavailability values (<1%) for the several different cereals studied, among other, bitter and sweet quinoa. [6] Additional data from human studies confirmed a 1.6-fold higher Fe absorption ratio from wheat than quinoa infant cereals [8] that results similar to the 1.1-fold higher Hb concentration found in ID animals fed with WWB in relation to QB.

An important variable, when monitoring iron absorption for different cereal grains, is the nature and phytic acid (or phytates) concentration of the sample. In this study it was observed significant differences in InsP_6 concentration between the different breads that were not reflected in significant changes in Hb. For example, amaranth flour provided 4.6-fold higher InsP_6 content than flour from chia (Table 1), but animals administered with bread formulations containing either amaranth or chia showed similar Hb concentrations (Table 2). However, both bread formulations improved the nutritional status of animals in relation to ID animals and those administered with white bread. These results contrast with the scarce studies existing where it is shown that supplementation with amaranth did not increased Fe absorption despite a large increase in iron intake (by 5.6-fold) and more favourable phytate:iron molar ratio (3:1 versus 5:1) than controls after an intervention period of 16-wk. [28,29] In the majority of published studies about the negative interaction of phytate on Fe bioavailability, there has been established a desirable ratio InsP_6/Fe of <0.4/1 as a critical value determining the inhibitory effect of phytate. [9] Thus, the addition of amaranth grain flour to maize-based porridge did not improve the nutritional Fe status in ID children. [7] *In vitro* studies have shown that can be defined a critical proportion of amaranth flour to be used in bread formulations favouring Fe bioavailabilty as indicated by the ferritin

concentrations quantified in cell cultures. [3] The bioaccessible phytate/Fe molar ratio rather than phytate content in the bread samples explained the amaranth flour-mediated inhibitory effect on the available Fe. There has been reported in the literature a great disparity in the concentration of phytates in amaranth grains (4.8-34.0 μmol/g). [3,30] In the present study, the amount of InsP_6 in the bread with 25% of amaranth was 3.7 μmol/g and seemed to exert low inhibitory effects on Fe bioavailability. Thus, allowing us to hypothesize that low InsP_6 concentrations are released during duodenal transit where it takes place most of micronutrient uptake. Additionally, *in vitro* studies evidenced that simple carbohydrates such as fructose can increase Fe uptake in human intestinal Caco-2 and HepG2 cells based on protein ferritin synthesis. [31] These studies are in line with recent experimental data demonstrating that structural differences of prebiotic fibres influence Fe uptake to ID animals. [12] Thus, studies in this line are advisable to understand the nutritional implications of their use in bread formulations given that pseudocereals have been suggested as healthier alternatives to white bread to better control the glycemic index. [32]

Inflammation is an important contributor to the development of chronic diseases. Thus, it was examined whether ancient Latin-American crops-containing bread formulations were associated with lower concentrations of inflammatory markers, which play a key role in intestinal Fe absorption and liver metabolism. The observation that pseudocereals-based bread formulations decreased plasmatic levels of hepcidin bioactive peptide (Fig. 2) suggests that the release from bread formulations of a high proportion of compounds able to chelate the micronutrient preventing the hepcidin-mediated inhibition of intestinal absorption. This suggestion is supported by the down-regulation of mRNA expression levels of TfR2 (Fig. 2) that serves as a body Fe sensor of plasmatic concentration of the micronutrient. [11,24] Decreased concentrations of TfR

have also been quantified in ID children even after they received a multi-micronutrient powder (2.5 mg Fe/day). [7] Experimental models as well as human studies have demonstrated the inverse relationship between serum hepcidin concentration and Fe absorption from food and supplemental sources. [10,11] The low inflammatory potential estimated for the pseudocereals is in line with the reported anti-inflammatory effects of amaranth [33] and quinoa [34] in THP-1 human-like and mouse RAW 264.7 macrophages. Anti-inflammatory effects of pseudocereals could be probably attributed to the production of bioactive peptides or released saponins during processing. Additionally, human studies demonstrated that quinoa is well tolerated by patients with inflammatory autoimmune-based intestinal disorders such as celiac disease. [35] The low inflammatory potential of pseudocereals is reflected in the low transcripts (mRNA) of IL-6 (Fig. 2) that can have important nutritional consequences. It has been previously reported an inverse association of IL-6 levels with plasma Fe concentration and transferrin saturation together with increased ferritin in the liver. [36] Recent human data demonstrated the critical influence of quantity and quality of dietary carbohydrates favouring increased concentrations of pro-inflammatory cytokines such as IL-6. [37]

5. Conclusions

Most of existing data supporting beneficial nutritional effects of pseudocereals are based on their high content of nutrients, among other minerals. Notably, Fe absorption studies from both experimental and human intervention trials indicate poor bioavailability of the micronutrient from cereals. The data reported herein, on the administration of different bread formulations to iron deficient animals, show differences in Fe bioavailability. Except WWB and QB, bread formulations made with amaranth (AB) or chia (ChB) improved the Hb concentrations in relation to the WB.

However, taking into account the high content of minerals in the pseudocereals or chia, the potential contribution of these whole flours would not differ considerably from that of wheat flour. Notably, these data point out that incorporation of functional ingredients like pseudocereals/chia in bread making strategies is associated with a positive inflammatory profile preventing the hepcidin-mediated inhibition of Fe uptake at intestinal level. Further studies and human trials are needed to gain a better understanding of the potential nutritional influence of the carbohydrates-Fe interaction when using these flours in bread making strategies.

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Table 1. Iron and phytates (InsP_6) content in breads formulated with flours from different origin. Values are expressed as mean ± standard deviation (n=3). [a-e] Different letters in the same column indicate statistical differences (P<0.05). WB, white bread; WWB, whole wheat bread; AB, bread with 25% amaranth flour; ChB, bread with 5% chia whole flour; QB, bread with 25% of quinoa whole flour.

Sample	Iron (μmol/g)	InsP_6 (μmol/g)	InsP_6/Iron [1]
WB	0.42 ± 0.05 [a]	n.d.	--
WWB	0.67 ± 0.03 [b]	2.57 ± 0.12 [b]	3.8>1
AB	0.77 ± 0.08 [c]	3.65 ± 0.38 [c]	4.7>1
ChB	0.48 ± 0.02 [a]	0.75 ± 0.03 [a]	1.6>1
QB	0.61 ± 0.01 [ab]	2.04 ± 0.04 [b]	3.3>1

[1] A maximum critical value of 1 has been established for the molar ratio InsP_6/Iron as inhibitory for Fe uptake (Hurrell, 2004).

Table 2. Haematological parameters in iron-deficient (ID) and animals fed with $FeCl_3$ alone or together with different bread samples[a].

			Treatment					
	Control	**ID**	**$FeCl_3$[b]**	**WB**	**WWB**	**AB**	**ChB**	**QB**
Haemoglobin (Hb, g/dL)	18.9 ± 0.5a	11.1±2.7b	16.2±0.2a	12.3±0.2b	14.7±1.3ab	16.4±2.2a	17.4±2.8a	13.4±2.9ab
Haematocrit	54.3 ± 2.8a	46.9±2.6a	51.2±2.5a	52.0±2.4a	49.0±3.9a	54.2±4.2a	55.9 ± 5.2a	61.3 ± 7.3a
MCV ($x10^{-4}$)	1.68±0.04a	1.64 ±0.04a	1.71±0.04a	1.69±0.04a	1.70±0.04a	1.97±0.04bc	1.91±0.04b	2.07±0.04c
MCH (pg)	33.7±2.1a	20.3±5.5b	28.9±0.3a	25.3±5.9ab	27.9±4.6ab	26.2±4.1ab	31.2±5.0b	21.9±4.8ab

[a]Values are presented as mean ± standard deviation (n=5). Values following by the same letter in the same line are not statistically different ($P<0.05$). WB, white bread; WWB, whole wheat bread; AB, bread with 25% amaranth flour; ChB, bread with 5% chia whole flour; QB, bread with 25% of quinoa whole flour; MCV, mean corpuscular volumen; MCH, mean corpuscular Hb; [b]$FeCl_3$ dosed at 2.5 μg

Figure 1. Plasma hepcidin (Hamp) peptide concentrations in iron-deficient animals (ID) and administered with $FeCl_3$ or breads formulated with different flours. Values are expressed as mean and range (n=5). * Indicates statistical differences (P<0.05) in relation to iron deficient animals (ID).

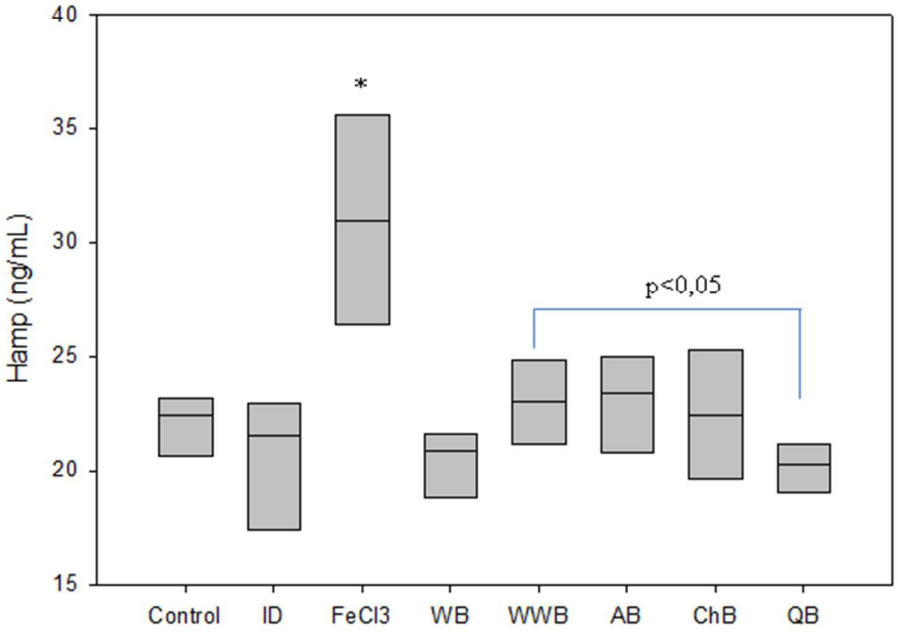

Figure 2. Hepatic expression of transferring receptor (TfR2) and interleukin (IL)-6 in iron deficient (ID) animals and those administered with a supplement of iron (FeCl3) alone or the different bread formulations (white wheat, WB; whole wheat, WWB; amaranth, AB; chia, ChB; quinoa, QB). Results are expressed as mean ± standard deviation (n=5). Superscript symbols indicate statistically ($p<0.05$) significant differences (*, TfR2; §, IL-6) relative to controls and between groups of treatment.