**Supplementary material**

**COLONIC AND SYSTEMIC EFFECTS OF EXTRUDED WHOLE GRAIN SORGHUM CONSUMPTION IN GROWING WISTAR RATS**

Llopart Emilce E.1, Cian Raúl E. 1, López-Oliva Muñoz María Elvira2, ZuletaÁngela 3, WeisstaubAdriana 3 and Drago Silvina R.1\*

**4-Systemic effects of sorghum diets**

**4.1-Lipid profile**

Consumption of dietary fiber induces both local and systemic responses. Higher activity of the GI microbiota elevate metabolism and absorption of the polyphenols derived from the diet and thus may exhibit systemic modulatory effects. In the present study, sorghum diets significantly decreased TAGs levels in serum (C: 0.84 (SEM 0.02), EWS: 0.31 (SEM 0.03), and ERS: 0.13 (SEM 0.01) g/L; *P*: 0.0001), being lower for ERS, but there was no difference between diets for cholesterol (C: 0.55 (SEM 0.05), EWS: 0.56 (SEM 0.04) and ERS: 0.46 (SEM 0.02) g/L (*P*: 0.1850). Thus, extruded sorghum intake might be beneficial for preventing hypertriglyceridemia. It may be associated with the changes in cecal fermentative processes, increasing the colonic concentrations of SCFA. Acetate and propionate are known to differentially influence fat metabolism; acetate is the primary substrate for cholesterol synthesis in the liver, whereas propionate has been reported to be involved in the cholesterol lowering effect (55). However, neither differences in the hepatic lipid profile were observed between the dietary groups (Table 4), showing that sorghum diets only induced a moderate changes on the lipid metabolism. In this sense, also no change in hepatic TAGs and cholesterol were observed in rats feed with WG wheat (56) or WG rice (57).

**4.2- Effect of sorghum diets on systemic oxidative status**

The liver is an important organ in which a number of metabolic reactions occur. Changes in other tissues to a certain extent also are reflected in the metabolic activity of the liver. Results of different indicators of oxidative status are showed in Table 4. In the present experiment, hepatic reducing power (RP) was higher for animals consuming both sorghum diets. Also, there were slight but significant differences for serum RP among animals consuming different diets (1.42 (SEM 0.06), 1.66 (SEM 0.06), and 2.25 (SEM 0.06) mg AA/g protein for C, EWS, and ERS diet, respectively) (*P<*0.0001).

There was no difference for CAT activity, but ERS more effectively increased the hepatic antioxidant capacity than the diet with EWS, with increased GR activity leading to scavenging toxic intermediates of reactive oxygen species. Both sorghum diets decreased GSSG levels respect to control, but resulting in not significantly different GSH/GSSG ratios. However, a trend was observed since ERS diet induced higher ratios than C. Many researchers propose that a high GSH/GSSG ratio can prevent the harmful effects of GSH depletion (58, 59). Thus, sorghum diets would exert a greater reducing effect on the overall liver redox state. This could be due to that during extrusion large molecular weight polyphenols might be depolymerized to monomers and dimers in response to the heat and shear, changing polyphenol bioavailability (60). Extrusion of cranberry pomace resulted in significantly increased in low molecular weight procyanidins and antioxidant capacity (61). However, these results were not reflected in the reduction of lipoperoxidation measured by MDA levels. Animals consuming EWS diet had higher hepatic MDA eq content, while rats fed with ERS did not shown changes in MDA levels respect to C. However, Albarracín et al. (57) reported lower liver MDA content in rats fed with extruded WG corn and rice respect to control. Also, differences were not observed (*P*: 0.1355) in serum MDA eq content of animals fed with different diets (C: 57.20 (SEM 4.19), EWS: 66.74 (SEM 1.89) and ERS: 66.54 (SEM 2.47) nmol/ g protein). Similarly, there was no difference by feeding rats with WG extruded maize (34) and whole rice (62). However, tea or polyphenol consumption significantly decreased in MDA eq serum or tissues in different animal models (63) and a procyanidin-rich extract from sorghum bran significantly repaired the D-galactose-induced oxidative damage in mice in both liver and serum (64). In this regard, Rezar et al. (65) stated that the AO effects of WG can more easily be seen in animals in pro-oxidative conditions. Animals consuming ERS-based diet showed higher antioxidant capacity and RP in both liver and serum than those of C and EWS, although it was not reflected in a lower content of MDA eq. It is possible that despite of extrusion, the low bioavailability of phenolic compounds only allows they mainly exert AO effects at colonic level and only modest systemic effects are observed, as they depend on polyphenols or their active metabolites absorption. Also other researchers awarded the lack of systemic AO effect of WG to the low polyphenol bioavailability (66).

**Table 4.** Triacylglycerols (TAGs), Cholesterol, malondialdehyde equivalent (MDA eq), Reducing Power (RP), Catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione (GSH) and glutathione disulfide (GSSG) in liver of rats fed with control (C), extruded white sorghum (EWS) and extruded red sorghum (ERS). \*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Diets** | **C** | **EWS** | **ERS** | **p-Value** |
| **Mean** | **SEM** | **Mean** | **SEM** | **Mean** | **SEM** |
| **TAGs (µmol/g TD)** | 26.55a | 1.78 | 30.37a | 0.73 | 26.45a | 1.48 | **0.2087** |
| **Cholesterol (mg/g TD)** | 5.64a | 0.30 | 6.65a | 0.16 | 5.74a | 0.34 | **0.1049** |
| **MDA eq (nmol/100 mg protein)** | 19.00a | 1.82 | 30.88b | 1.47 | 20.90a | 3.14 | **0.0001** |
| **RP (mg AA/g protein)** | 20.88a | 2.00 | 35.74b | 2.39 | 42.00c | 1.13 | **0.0000** |
| **CAT (μmol/seg g protein)** | 8.79a | 0.07 | 8.86a | 0.24 | 8.64a | 0.38 | **0.8264** |
| **GR** **(nmol NADPH/min/mg protein)** | 28.27a | 1.42 | 32.25a | 2.16 | 39.76b | 1.96 | **0.0098** |
| **GPx** **(nmol NADPH/min/mg protein)** | 716.55a | 11.16 | 779.92a | 48.68 | 914.96a  | 78.83 | **0.1952** |
| **GSH (µg GSH/g TD)** | 2374.3a | 454.3 | 1733.8a | 219.3 | 1994.7a | 223.8 | **0.3402** |
| **GSSG (µg GSSG/g TD)** | 1039.1b | 84.3 | 548.6a | 47.6 | 604.3a | 75.2 | **0.0008** |
| **GSH/GSSG**  | 2.47a | 0.74 | 2.86a | 0.50 | 3.70a | 0.29 | **0.2492**  |

Data are expressed as mean ± SEM (n = 8 per group). Different letters mean significant differences between samples analyzed by the LSD test (p<0.05). AA: ascorbic acid, TD: Tissue damp.