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# EVALUATION OF THE PROTECTIVE EFFECT OF VITAMIN D ON MATERNAL AND FETAL PARAMETERS, HEPATIC AND RENAL FUNCTION OF WISTAR RATS SUBMITTED TO DIET WITH FRUCTOSE DURING PREGNANCY

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**Abstract: Goal:** to determine protective aspects of vitamin D (Vit.D) on maternal and fetal markers, liver and kidney function, as well as oxidative stress parameters of Wistar rats exposed to fructose ingestion throughout pregnancy. **Method:** After confirmation of pregnancy, the animals were divided into control (C) and treated groups. Group 40% fructose added to the ration; group 40% + Vit.D; group 30% and 30% + Vit.D. Vit.D was administered orally (via gavage) for 19 days, 20IU/kg/day. Results: In general, the main statistical data of the study show a more pronounced change in the number of fetuses for the 40% group ($p<0.01$). On the other hand, for the groups with the lowest fructose concentrations we obtained significantly higher mass ($p<0.001$). Biochemical data show changes in uric acid and blood glucose ($p<0.01$); urea, creatinine, triglycerides and total cholesterol ($p<0.001$). Liver function tests showed changes for the enzyme aspartate aminotransferase [AST] ($p<0.01$) and Alanine aminotransferase [ALT] ($p<0.001$). As for markers of oxidative stress, TBA-RS for liver and kidney tissue, the statistical significance was ($p<0.001$). Glutathione peroxidase (GSH) showed alteration ($p<0.05$) for renal tissue. **Conclusions:** The results showed changes in some fetal parameters, disorders of lipid metabolism, glycemic, liver and kidney function, as well as oxidative stress, becoming more pronounced for the groups in which the fructose concentration was higher and the addition of Vit.D was not adequate to maintain the redox balance.

**Keywords:** Fructose; Toxicity; Gestation; Research with animals, Nursing.

## INTRODUCTION

Fructose is a monosaccharide composed of six carbons joined in single covalent bonds containing a hydroxyl group and a carbonyl group (NELSON; COX, 2011). It is an important carbohydrate found in the organisms of animals and in most plants, having been isolated for the first time in 1847 from sugarcane (WANG, 1981). As a component of fruits and other vegetables, it is regularly ingested as a constituent of the diet, in addition to being synthesized by the body through the action of the sucrose enzyme, originating two monosaccharides (glucose and fructose), being absorbed by the transporter (GLUT 5) independent of insulin (SMITH). et al., 2005). The metabolism of this carbohydrate is carried out predominantly in the liver and to a lesser extent in the proximal epithelium of the renal tubules and in the small intestine (BARREIROS et al., 2005).

In animal and human studies, an increase in triglycerides and uric acid has been demonstrated after the ingestion of diets containing high concentrations of fructose as well as an increase in lipogenic enzymes. The increase in the activity of these enzymes results in greater lipid synthesis and consecutively high levels of these metabolites in the circulation, in addition to very low density lipoproteins (BOUTELDJA; TIMSON, 2010).

The physiologically relevant lipids are phospholipids, consisting of cholesterol, triglycerides (TG) and fatty acids (FA). These fats are important for constituting the framework of cell membranes. In addition to this function, cholesterol is a precursor of steroid hormones, bile salts and vitamin D, also acting on the fluidity of cell membranes by activating enzymes anchored in this structure. TGs are formed from fatty acids attached to a glycerol molecule. They are important for providing the deposit of muscle adipose tissue, considered one of the forms of elementary energy storage (MOSCA, 2011; XAVIER, 2013).

An important metabolite of fructose is uric acid and the excess (<7mg/dl) characterizes hyperuricemia. The pathogenesis of

hyperuricemia consists of the end product of purines that are synthesized from non-purine precursors derived from the degradation of nucleic acids of exogenous or endogenous origin, mediated by the enzyme hypoxanthine guanine phosphoribosyl transferase. Some researchers suggest that elevated uric acid passes into the fetal circulation and is likely to cause maternal and fetal death (ANDREW, 2005).

Oxidative stress was initially defined as "an imbalance between oxidant production and antioxidant capacity" (HALLIWELL, 2012). On the other hand, not contradicting the above definition, Jones (2006) offered another definition inferring that redox imbalance can occur due to "an interruption of the redox signal and control". This last definition, according to the same source, could be more adequate to justify oxidative stress during the aging process, where GSH levels decline and this evidence supports the concept that oxidative stress increases with aging.

The redox imbalance, regardless of the concept to be used, can be quantified in humans and animals through GSH/ glutathione disulfide (GSSG). Plasma GSH in humans becomes oxidized with age and in response to stress from human attitudes and behavior such as smoking and in chronic non-communicable diseases such as type 2 diabetes mellitus and cardiovascular disease (ROEDE et al., 2013).

Redox reactions are necessary for normal cell function and the chemicals generated are also used as signaling molecules for important physiological functions. These are continuously synthesized and used by antioxidant defense systems. However, when produced in high concentrations or when antioxidant defenses are deficient, this redox imbalance can cause cellular damage. If the increase in reactive species is relatively small, the antioxidant response will be sufficient to compensate for this increase (Sies, 1985). However, under certain pathological conditions or functional disorders, the production of reactive species is markedly increased and antioxidant defenses may be insufficient to restore redox homeostasis (FINKEL, 2000). The disruption between the pro-oxidant and antioxidant balance characterizes oxidative stress, and may represent a fundamental mechanism for the generation of human diseases, especially chronic pathological processes (SCHAFER, 2001; HALLIWELL and GUTTERIDGE, 2007;).

Thus, the term "oxidative stress" is used to define the situation in which the generation of reactive species exceeds the capacity of available antioxidant defenses. It can result either from reduced antioxidant defenses or increased production of oxidants, as well as the release of transition metals or a combination of any of these factors (HALLIWELL, 2012). The synthesis of reactive oxygen (ROS) and nitrogen (RNS) species is an integral part of metabolism as well as necessary for functions such as phagocytosis (VERCESI et al., 2018).

Free radicals play an important role in cells, so an increase in the formation of free radicals can be beneficial, as is the case with the release of toxic oxidant species by neutrophils, which can act in the host's defense against infections. On the other hand, the beneficial effects of antioxidants have been extensively studied (DELANTY and DICHTER, 1998; LIU, 2019). They also participate in cell signaling processes and are also involved in the synthesis and regulation of some proteins (HALLIWELL; GUTTERIDGE, 2012).

On the other hand, when formed in excess, these highly reactive species have the potential to oxidize biomolecules including proteins, lipids, DNA and RNA (DROGE; 2002; HALLIWELL; GUTTERIDGE, 2012; SHARIFI-RAD, 2020). Regarding the harmful effects of oxidative reactions to the body,

free radicals can promote lipid peroxidation, mainly of polyunsaturated fatty acids from cell plasma membranes; oxidation of low density lipoproteins (LDL); react with proteins, leading to their inactivation and consequent alteration of their function; and react with DNA and RNA, leading to somatic mutations and transcription disorders, among other effects (DELANTY; DICHTER, 1998).

To counteract damage caused to cells, there are antioxidant enzymes in which endogenous or exogenous substances that reduce the formation of free radicals or react with them, neutralizing them. Thus, under conditions of high production of reactive species, the cell can protect itself against possible oxidative damage through non-enzymatic and enzymatic antioxidants. Although differing in composition, antioxidant defenses are widely distributed in the body and comprise agents that catalytically scavenge free radicals, such as the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), among others. (HALLIWELL; GUTTERIDGE, 2012).

The Vit. D acts as a steroid hormone, whose main function is to regulate calcium and phosphorus homeostasis, bone formation and resorption, in addition to interacting with the parathyroids, kidneys, intestines, thyroid and liver (TOMEDI et al., 2013). As it constitutes a prohormone, to be activated, it undergoes hydroxylations: initially in the liver, forming 25-hydroxyvitamin D (25-OHD3), called calcidiol; subsequently in the kidneys, it forms two main metabolites: 1α,25-dihydroxyvitamin D, known as calcitriol, and 24R,25-dihydroxyvitamin D3, also known as 24-hydroxycalcidiol (CHIELLINI, 2011; WACKER, 2013). The regulation of vitamin D through the endocrine system occurs in the kidney, through the control of the activity of the 1-hydroxylase enzyme. In this case, the synthesis of calcitriol is carried out considering calcium concentrations and metabolic needs.

Due to its versatile physiological characteristics, its elementary function is to maintain cellular homeostasis. It acts as a component of cell signaling, maintaining the redox balance in relation to reactive oxygen species (ROS). This stability depends on the ability of vitamin D to control the expression of these components acting to reduce ROS levels (HOLLIS, 2005). Regulatory deficiency of vitamin D can accelerate the aging process, expressed by a decline in cognition in rats that can be reversed by administration of vitamin D. Deficiency of this vitamin has also been associated with two of the major diseases in man: heart disease and heart disease. Alzheimer's (BOUILLON et al., 2008).

Vitamin D is an essential micronutrient, mainly important for calcium absorption and consequent calcification of bone tissue. The compounds that form vitamin D are fat-soluble and the main structure of this group is to have an isopropene unit that is formed by the opening of a ring of cyclopentaneperhydrophenanthrene (cholesterol), and the active compounds of vitamin D are ergocalciferol and cholecalciferol ( LI et al., 2004).

Vitamin D in addition to having a function of direct calcification of bones, concomitantly acting as a signal for other cells that have a specific receptor such as kidney cells, pancreatic, hematopoietic, epidermal, neurons and muscles, indicating other biological functions not directly related to calcium. In general, the amount of vitamin D obtained from the diet varies between 100 IU and 200 IU per day. Vitamin D compounds and their metabolites are mainly excreted in bile and feces and in small amounts in urine.

Vitamin D deficiency has been linked to systemic arterial hypertension and cardiovascular disease (DONG, et al., 2014). Vitamin D's ability to protect the cardiovascular

system may depend on its ability to maintain the stability of reactive oxygen species (ROS) and Ca2 signaling systems, which are known to be decompensated in hypertension, cardiac hypertrophy, congestive heart failure (CHF) ) and disturbances in the electrical function of the heart, causing arrhythmias. One of the main causes of cardiac hypertrophy is hypertension and the renin-angiotensin system (RAS) plays an important role in blood pressure regulation (HAYES; DINKOVA-KOSTOVA, 2014; BENCE; DONALD; BOSSUYT, 2016). One of the primary actions of vitamin D is to reduce RAS function to prevent hypertension which is a major risk factor for heart disease (WENG et al., 2013).

In rats, the deletion of the enzyme 25 (OH) D1 α-hydroxylase (controller of renin secretion) resulted in increased stimulation of the renin-angiotensin system. Consecutively, an increase in blood pressure levels and onset of cardiac hypertrophy were observed (ZHOU et al., 2008). On the other hand, in patients with type 2 diabetes and associated hypertension, the hypertensive condition was improved after vitamin D supplementation (NASRI et al., 2014). Excessive renin release and the resulting increase in angiotensin II can have multiple effects on some of the key components of the cardiovascular system. Under these conditions, the action of angiotensin II increases the formation of endothelin-1, which is a potent vasoconstrictor and thus contributes to angiotensin II-induced hypertension (ORTIZ et al., 2001).

On the other hand, Vit. D is also identified as a signaling factor for the synthesis of antioxidants (BERRIDGE, 2015). This way, BHATTACHARYYA (2014) describes that to Vit. D is related to cellular oxidative stress, due to its antioxidant properties. Lin (2005) points out that changes in the redox balance can lead to oxidative stress damage and, this way, lead to the dysfunction of various cellular processes, sensitive to oxidation. According to the aforementioned reference to Vit. D exerts protective effects against reactive oxygen species, giving it the potential to prevent cellular oxidative damage. When there is an increase in reactive oxygen species levels in response to cellular stress, erythroid nuclear factor 2 binds to the nucleus acting as an antioxidant response element. In this situation, the cellular response to redox imbalance occurs by increasing levels of antioxidants such as catalase (CAT), Reduced Glutathione, GPX and SOD (LEE et al., 2012; TSAI et al., 2011). This way, vitamin D can modulate oxidative stress, being able to exert several beneficial contributions in the body, since oxidative stress is related to pathological conditions (LIGUORI et al, 2018)

To answer some gaps and interfaces of the effects of fructose ingestion in pregnant rats, the experiment aimed to determine protective aspects of Vit. D on hepatic and renal tissue oxidative stress parameters in addition to the determination of aspects of maternal and fetal toxicity of Wistar rats exposed to fructose ingestion throughout pregnancy.

## METHODOLOGY

This is an experiment in which 50 pregnant female rats (Rattus norvegicus) from the URI vivarium were used, distributed in the control and treated groups. The animals were housed in standard cages containing a maximum of four animals in each unit, kept in 12-hour light-dark cycles, at a temperature of 23±1°C, with free access to exhaust, food and water. During this period, necessary care was also taken with the animals, such as feeding, cleaning cages and drinking fountains.

The present research had an in vivo experiment, in which virgin females were mated with males of the same species and pregnant women were detected by collecting material from the vaginal canal through a

smear, arranged on slides and visualized under an optical microscope where the pregnant women were identified through the visualization of sperm. From this moment on, the matrices were separated from the males, divided into 5 groups with ten cobials each. The control group (C) received standard chow; group (40%) received this concentration of fructose added to the diet; group (40%+Vit.D) received the same diet and with the addition of Vit.D 20UI/Kg/day (RAMOS, 2016). The group (30%) received this concentration of fructose added to the ration and the group (30%+Vit.D) received the same concentration of fructose to the ration and with supplementation of Vit.D 20UI/Kg/day, through gavage. The addition of fructose was formulated manually and the Vit.D was administered by gavage. The project was approved by the CEUA of URI Erechim.

On the 19th day of gestation, the sows were euthanized, initially with the administration of Zoletil 50mg/Kg (i.p.) and after exsanguination by cardiac puncture, as recommended by the Guidelines for the Practice of Euthanasia of the National Council for the Control of Animal Experimentation - CONCEA, published through of Normative Resolution No. 13, of September 20, 2013 (BRASIL, 2013). At this moment, a macroscopic analysis of the maternal structures, the fetuses, as well as their weight was performed. Subsequently, blood was collected through intracardiac puncture for further analysis of biochemical parameters. This tissue was centrifuged to obtain plasma for the determination of biochemical parameters performed with commercial Labtest® kits.

From plasma, the levels of uric acid, blood glucose, triglycerides, total cholesterol, creatinine, aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined. From liver and kidney tissue parameters of oxidative stress were determined.

Subsequently, perfusion was performed by infusing saline into the systemic circulation to remove blood before harvesting liver and kidney tissue for oxidative stress testing. These tissues were packed in ependorfs and kept in a -80ºC freezer for further analysis. At the time of analysis, these structures were homogenized (1:10 w/v) in sodium phosphate buffer (200 mM) with KCl (140 mM). The homogenate obtained was centrifuged at 3000 rpm for 10 min and the supernatant separated for redox homeostasis techniques. For these tests, the supernatants obtained from the different tissues were incubated at 37 ºC in the presence of organic acids for 1 hour. The measurement of levels of thiobarbituric acid reactive substances (TBA-RS) was performed according to the ESTERBAUER method; CHEESEMAN (1990). Measurement of Reduced Glutathione Levels (GSH) was performed according to the method of (SALAT et al., 2014).

The results will be presented as mean and standard error and for analysis of variance a one-way ANOVA (One ANOVA) followed by Tukey's post hoc test of multiple comparisons was used. Values of $p<0.05$ were considered statistically significant.

## RESULTS

Initially, the results of the initial and final weight of the sows, the number and weight of the fetuses at the 19th day of gestation will be shown. Subsequently, the results of biochemical tests will be presented, followed by markers of oxidative stress in kidney and liver tissue. The results will be presented through the mean and standard error (Avg±Ep) and the statistical significance when applicable.

The study data show that in the groups treated with fructose the average number of fetuses was lower than the control group,

**Number of fetuses**

| C | 40% | 40%+Vit.D | 30% | 30%+Vit.D |
|---|---|---|---|---|
| 10,67±1,75 | 5,33±3,67 | 9,80±1,64 | 9,14±1,57 | 6,78±3,93 |

**Mass of fetuses**

| C | 40% | 40%+Vit.D | 30% | 30%+Vit.D |
|---|---|---|---|---|
| 1,54±0,2g | 0,79±0,05g | 0,99±0,06g | 2,18±0,2g | 1,81±1,7g |

Data are expressed as mean ± standard error for 10 animals per group.

Table 1 – number and mass of fetuses.

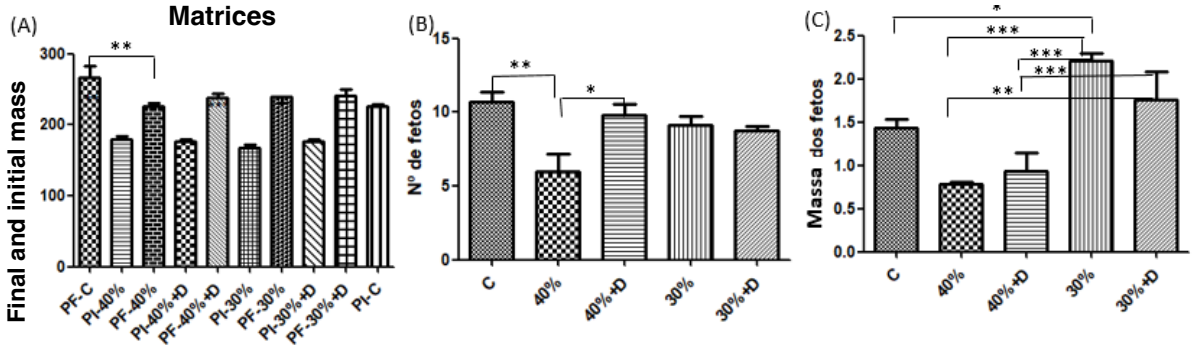


Figure I – Effects on maternal-fetal parameters determined by fructose intake at concentrations of 40 and 30%, with or without Vit supplementation. D during the gestation period of Wistar rats. (A) initial and final mass of the matrices. PF-C= final mass of the control group, PI-40%= initial mass of the 40% group; PF-40%= final mass of group 40%; PI-40%+D = initial mass of the 40% group + Vit. D 20IU/Kg/day; PF_40% +Vit.D = final mass of the group 40% + Vit. D 20IU/kg/day; PI 30% initial mass of the 30% group; PF-30%= final mass of group 30%; PI -30% +D = initial mass of the 30% group with addition of Vit. D 20IU/kg/day; PF-30% +D = mass of group 30% with addition of vit. D 20UI/Kg/day and PI-C= initial mass of the control group. (B) number of fetuses: C= number of fetuses in the control group; 40%= number of fetuses in the 40% group; 40% +D = number of fetuses in the 40% group with addition of Vit. D 20IU/kg/day; 30%= number of fetuses in the 30% group; 30% +D = number of fetuses in the group treated with 30% fructose with addition of Vit. D 20IU/kg/day. (C) Mass of fetuses: C = mass of fetuses in the control group; 40%= mass of fetuses in group 40%; 40% + D= mass of fetuses in the 40% group with addition of Vit. D 20IU/kg/day; 30%= Mass of fetuses in group 30%; 30% +D = mass of fetuses in group 30% with addition of Vit. D 20IU/kg/day. Parameters obtained from Wistar rats, in groups of nine animals with a normal diet for the control group and the others with the same number and with diet (40% and 30% of the addition of fructose to the diet; with and without supplementation of Vit.D ) during the gestation period. Data represent mean ± standard error and statistical calculation was performed using analysis of variance (ANOVA), followed by Tukey's post-hoc test and statistical significance was defined *p<0.05; **p<0.01; ***p<0.001.

being more pronounced for the 40% group. Likewise, the fetal mass was lower for the 40 and 40%+Vit.D groups when compared to the control. On the other hand, the 30% and 30%+Vit.D groups had a mass higher than the average of the control group.

The analysis of the initial and final weight of the sows (figure I A) shows that all sows obtained mass gain during pregnancy, with a statistically significant difference $p<0.01$ for the final mass of the animals in the control group with the final mass of the group 40 %. On the other hand, considering the number of fetuses (figure IB), it also showed a statistically significant difference ($p<0.01$) when comparing the control group with the 40% group. The 40%+Vit.D group, compared to the 40% group, had an increase in mass, with a statistically significant expression ($p<0.05$). As for the fetal mass (figure IC), the study showed a statistically significant difference $p<0.05$ between the control group and the 30% group, showing a considerable increase in the mass of this group ($p<0.001$) compared to the 40% and 40%+ D Vit groups. The same statistical pattern was evidenced for the 30%+Vit.D group compared to the 40%+Vit.D group. The data also showed statistical significance $p<0.01$ between the 30%+Vit.D group and the 40%+Vit.D group.

The table above represents the average of the results of the biochemical data. In this sense, the average uric acid rate for all groups treated with fructose obtained higher values when compared to the control group, being more pronounced for the 30%+Vit.D group. Using the same parameters, all groups treated with fructose had higher glycemia rates when compared to the control group, with emphasis on the 40%+Vit.D and 30% groups. Urea rates were also higher for all groups, drawing attention to the group. Another important aspect to be observed and that calls attention is the creatinine levels, higher in all groups when compared to the control. This detail is relevant given the short time the guinea pigs were submitted to this diet, as well as the physiological action of renal function and its repercussions when these organs are unable to maintain homeostasis. Worthy of note are the levels of triglycerides, seen by the extreme difference when the data are compared to the control group.

The analysis of biochemical parameters, the study showed a statistically significant difference in uric acid rates ($p<0.01$) of the control group compared to the 30%+Vit.D group and $p<0.05$ of this compared to the 40%+Vit.D group (figure II). Blood glucose rates (figure IIE) showed a statistically

| Groups | Uric acid | Blood glucose | Urea | Creatinine | TG. | Total Cholesterol | AST | ALT |
|---|---|---|---|---|---|---|---|---|
| C | 1,42±0,19 | 118,17±2,48 | 40±3 | 0,66±0,04 | 41,67±12 | 42±5,51 | 110,83±83 | 65,67±6,4 |
| 40% | 1,76±0,23 | 138±24,76 | 43,6±5,5 | 0,81±0,09 | 230±99,4 | 60,33±13 | 154,4±44,3 | 75,11±10 |
| 40%* | 1,51±0,21 | 163,25±41,1 | 50,3±15,1 | 0,84±0,14 | 225±88,6 | 87,5±14,8 | 137,2±29,1 | 75,1±14,4 |
| 30% | 1,71±0,3 | 179,11±25,2 | 83,8±6,94 | 0,83±0,07 | 233,3±62 | 83±2,83 | 122,4±10,2 | 83,5±8,41 |
| 30%* | 1,93±0,34 | 152±37,03 | 48,5±12,9 | 0,68±0,1 | 292±90,1 | 66,3±16 | 86,44±9,5 | 72,4±5,8 |

Data are expressed as mean ± standard error for 10 animals per group, * (+Vit.D).

Table 2. Levels of uric acid, blood glucose, urea, creatinine, triglycerides, total cholesterol, AST, ALT of the mothers.

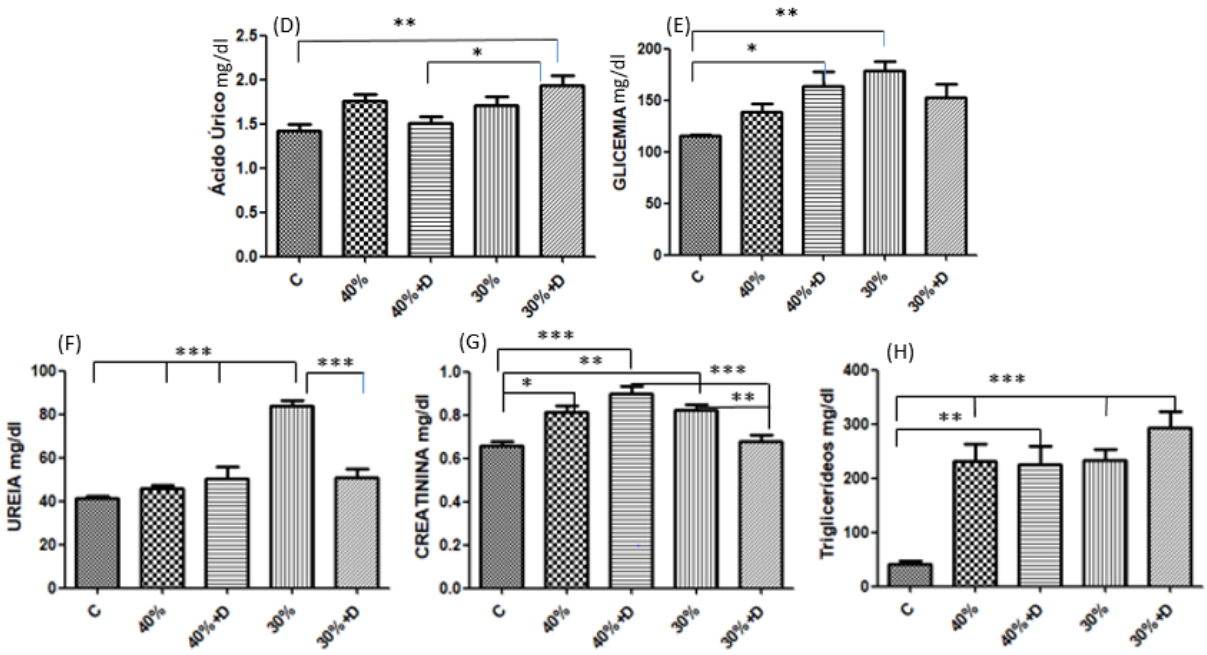


Figure II - Effect of fructose ingestion alone or with Vit supplementation. D (20UI/Kg/day) of Wistar rats on markers of renal, hepatic, lipid and glycemic metabolism. Renal function was estimated through urea and creatinine rates. For lipid metabolism, total cholesterol and triglycerides were used. Liver function was estimated using AST and ALT enzymes. (D) serum uric acid levels; (E) Serum blood glucose levels; (F) Serum urea levels; (G) Serum creatinine levels; (H) Serum levels of triglycerides. Parameters obtained from nine Wistar rats, with a normal diet for the control group "C" and the others with a diet (40%; 40%+Vit.D; 30% and 30% +Vit.D) of fructose added to the diet during the gestation period.. Data represent mean ± standard error and statistical calculation was performed using analysis of variance (ANOVA), followed by Tukey's post-hoc test and statistical significance was defined *p<0.05; **p<0.01; ***p<0.001.

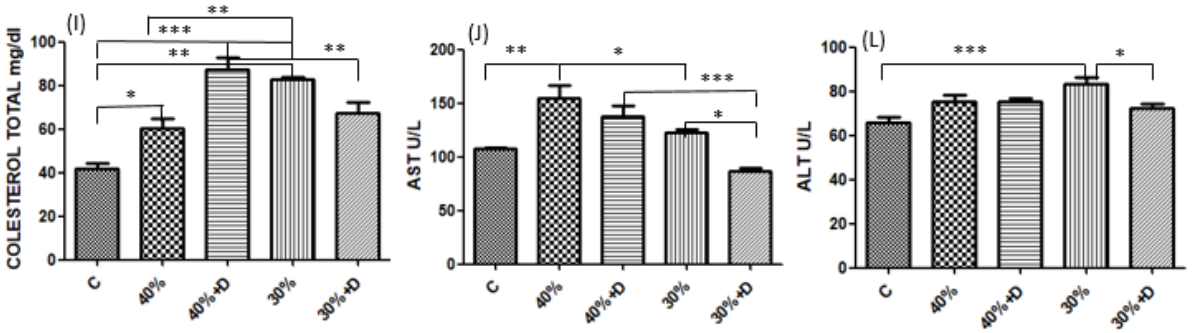


Figure III - Effect of fructose ingestion alone or combined with Vit.D of Wistar rats on markers of liver function, lipid metabolism. For lipid metabolism, total cholesterol and triglycerides were used. Liver function was estimated using AST and ALT enzymes. (I) Serum total cholesterol levels; (J) Serum AST levels; (L) Serum ALT levels. Parameters obtained from nine Wistar rats, with a normal diet for the control group "C" and the others with a diet (40%; 40% +Vit.D; 30% and 30% +Vit.D) of fructose added to the diet during the gestation period.. Data represent mean ± standard error and statistical calculation was performed using analysis of variance (ANOVA), followed by Tukey's post-hoc test and statistical significance was defined *p<0.05; **p<0.01; ***p<0.001.

significant increase ($p<0.01$) in the control group vs 30% and $p<0.05$ in the control group vs 40%+Vit.D. The urea rates (figure IIF) were high and with statistical significance $p<0.001$ when comparing the control group with 40%, 40%+Vit.D and 30%. The 30% group compared to the 30%+Vit.D group also showed a statistically significant change similar to that shown above. Creatinine rates (figure IIG) showed changes for all groups: ($p<0.05$) in the control group vs 40%, ($p<0.001$) in the control group vs 40%+Vit.D, ($p<0.01$) of the control group vs 30%. There were also statistically significant changes in the comparison between the 40% +Vit.D vs 30%+Vit.D groups ($p<0.001$) and ($p<0.01$) in the comparison of the 30% vs 30% +Vit.D. The levels of triglycerides (figure IIH) were high ($p<0.001$) when comparing the control group vs 40% and 30%+Vit.D and between the control group and 40%+Vit.D (p<0, 01).

Total cholesterol rates (figure III-I) showed changes for all groups: ($p<0.001$) control group vs 40%+Vit.D and 30%, control vs 30%+Vit.D ($p<0.01$), Control vs 40% ($p<0.05$). Additionally, it also showed noteworthy alterations for the 40% and 30% groups ($p<0.01$). Liver function was determined by determining AST and ALT rates. This (figure III-J) showed changes in the control group vs. 40% ($p<0.01$) and with the 30% group, $p<0.05$). The data also showed changes in the 40%+Vit.D vs 30%+Vit.D group ($p<0.001$) and with the 30% group ($p<0.05$). The ALT enzyme (figure III-L) showed statistical changes when comparing the control group vs 30% ($p<0.001$) and this vs 30%+Vit.D ($p<0.05$).

Regarding oxidative stress, TBA-RS rates in liver tissue showed high levels for all groups. From these data it is possible to infer that the diet defined in the protocol of the current study caused lipid peroxidation, being more pronounced for the 30% group. On the other hand, in renal tissue we did not observe the same pattern of change. GSH rates in liver tissue showed a reduction in all groups when compared to control, becoming more pronounced for the 40% group. A possible explanation for the fact that in the groups treated with fructose the concentrations of GSH were more reduced is the consumption of this antioxidant to face the excess of oxidants synthesized from the ingestion of fructose, not being sufficient to maintain the redox balance, in this tissue. On the other hand, in renal tissue the data showed important changes for the 40% group when compared to the control group.

In the current study, in liver tissue, TBA-RS (figure IV-M) showed statistically significant changes for all groups when compared to control: group C vs 40% and 40%+Vit.D ($p<0.001$); C vs 30% ($p<0.001$). Additionally, the results also showed changes between the 30% vs 30% +Vit.D group ($p<0.001$). These results possibly show a protective effect of Vit.D on this tissue at 30% fructose concentration. On the other hand, the results of GSH in the liver tissue show changes considered statistically significant in group C vs 40% ($p<0.05$). The reduced bioavailability of this chemical compound can be attributed to excessive consumption for the maintenance of redox homeostasis.

In renal tissue TBA-RS showed changes with the statistical significance established by the methodology of group C vs 40% ($p<0.05$) and C vs 30%+Vit.D. ($p<0.001$). Using the same statistical criteria, GSH showed changes in group C vs. 40% ($p<0.05$) and in this group vs. 40%+Vit.D ($p<0.05$). In this case, possibly indicating a protective effect of Vit.D on this tissue and at this concentration.

## DISCUSSION

In the present study, through the data presented, the influence of fructose on

| | TBA-RS (Hepatitis technician.) | GSH (Hepatitis technician) | TBR-RS (Renal Technician) | GSH (Renal Technician) |
|---|---|---|---|---|
| C | 0,21±0,06 | 37,20±9,58 | 1,21±0,56 | 11,81±7,6 |
| 40% | 0,28±0,05 | 23,61±3,77 | 0,86±0,22 | 13,08±4,8 |
| 40%+Vit.D | 0,30±0,08 | 24,95±4,84 | 0,63±0,14 | 9,88±3,99 |
| 30% | 0,44±0,08 | 26,36±7,04 | 0,72±0,2 | 8,99±1,33 |
| 30%+Vit.D | 0,28±0,04 | 29,71±9,38 | 0,48±0,07 | 8,42±2,44 |

Data are expressed as mean ± standard error for 10 animals per group.

Table 3. Levels of TBA-RS and GSH of hepatic and renal tissue, respectively, of the dams, 19 days after the start of fructose ingestion.

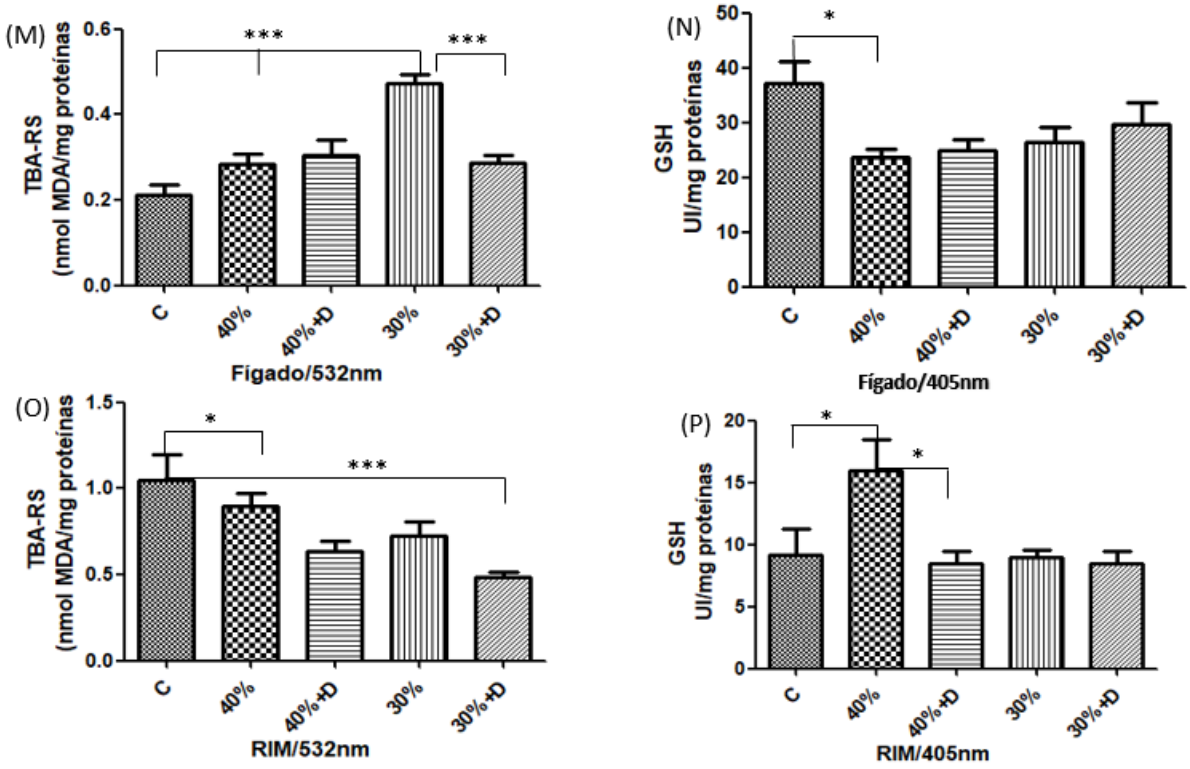


Figure IV - Effect of fructose ingestion alone or with Vit supplementation. D of Wistar rats on TBA-RS (M) and GSH (N) markers in liver tissue and kidney tissue (O) TBA-RS; (P) GSH. Parameters obtained from nine animals per group with normal diet for the control group "C" and the others with diet (40%; 40%+D; 30% and 30% +Vit.D) of fructose added to the ration during the period of gestation.. Data represent mean ± standard error and statistical calculation was performed using analysis of variance (ANOVA), followed by Tukey's post-hoc test and statistical significance was defined *p<0.05; ***p<0.001.

gestational and fetal parameters was characterized. The low final mass for the 40% group compared to the control may be indicative of maternal toxicity and the addition of Vit.D did not maintain the redox balance. The diet with added fructose significantly affected the number of fetuses mainly for the 40% group and, in this sense, the number of fetuses for the 40%+ Vit.D group was similar to the control, possibly indicating a protective effect on the generation of species reactive oxygen cells assigned to Vit.D for this group.

The body mass conditions of the fetuses, mainly in the 40% and 40% +Vit.D groups, contrasted with the mass of the fetuses in the 30% and 30% +Vit.D groups. In the case of fetuses, the body mass ratio is an important determinant for later growth and development. These findings were more pronounced for the groups where the concentration of fructose added to the diet was higher, possibly indicating that the effects are dose-dependent. These data, correlated to human pregnancy, gain relevance since low birth weight is statistically associated with increased morbidity and mortality and delay in growth and development. The reduction in the number of fetuses shows a deficit in fecundity and, in this situation, it may be related to maternal toxicity, which determined metabolic disorders, oxidative stress or both.

The low body mass and number of fetuses in the current study can be explained, at least in part, by the transfer of fructose from the mother to the fetus, driven by the GLUT 5 protein present in the placenta (SHAH, 1999). Increased rates of fructose in newborns, higher than maternal rates, were found by Barreiros (2006), indicating that this carbohydrate can be transferred via the placenta.

In general, considering the results from the biochemical tests, the study showed disturbances in hepatic metabolism, exposed by liver function tests, lipid metabolism, evidenced by cholesterol and triglycerides, disturbances of glycemic homeostasis indicated by glycemic data in addition to of the redox state disorder in the target tissues of the study.

Seen by the study design, the judgment of the results must be considered since the availability of the diet does not necessarily mean that the product has been ingested with the same volumes and intensity for all animals. These aspects may explain, at least in part, some conflicting results such as those found in the mass of fetuses, alterations in the enzymes used for liver function tests and some aspects of oxidative stress. According to Crescenzo et al., (2015) the ingestion of considerably high concentrations of fructose, in animals and humans, showed markedly high levels of triglycerides. Additionally, other undesirable effects attributed to a high-fructose diet in mice were associated with the development of obesity, which is characterized by adipocyte hypertrophy and low-grade chronic inflammation (GAMBARO et al., 2018), not explored in the current study.

The effect of high concentrations of fructose available in the diet of sows showed a reduction in fertility and in the number of fetuses (SABEN et al. 2016). The same authors describe that high concentrations of fructose affect pregnancy by altering the endometrium, determined by the reduction in the synthesis of steroid hormones and by factors that alter redox homeostasis, in favor of oxidative stress, possibly making implantation difficult. According to Malik et al (2019), the consumption of sweetened beverages was associated with morbidity and mortality mainly from cardiovascular diseases, associated with the volume ingested.

Ojeda et al. (2018), conducted an experiment with rats exposed to fructose ingestion during pregnancy and lactation. Some of the results of this study are similar

to the current study, where changes in the expression of liver enzymes, changes in the oxidative balance and metabolic profile of the matrices were observed. Additionally, the aforementioned reference describes the occurrence of metabolic syndrome, affecting pregnant mothers and later lactating mothers, determining an imbalance in the metabolic profile of the offspring. These findings were obtained from the offspring of sows exposed to a high fructose diet (65%). They conclude that exposure to fructose as a nutritional constituent during pregnancy alters the protein profile, leading to changes in redox homeostasis and some aspects of offspring metabolism.

Fructose ingestion can lead to hypertriglyceridemia, a significant increase in low-density lipoprotein (LDL-C), weight gain, systemic arterial hypertension and glucose intolerance, in addition to increasing oxidative stress HUANG et al. (2016). Similar results were also described by Zin et al. (2021). Additionally, this study also showed changes in the aspartate aminotransferase and alanine aminotransferase enzymes.

According to Yang et al. (2011) fructose ingestion may be associated with the pathogenesis of metabolic syndrome, including insulin resistance, abdominal obesity, dyslipidemia, intra-abdominal fat accumulation, hepatic steatosis, inflammation and endothelial dysfunction, resulting in type 2 diabetes mellitus. fructose and salt led to systemic arterial hypertension, possibly induced by the generation of reactive oxygen species and stimulation of renin secretion, which could contribute to an increase in systemic blood pressure figures (ZENNER et al. 2018).

Regarding oxidative stress, data from the current study show statistically significant changes in TBA-RS in liver tissue for all groups when compared to control, becoming more pronounced for the 30% $p < 0.001$ group, characterizing lipid peroxidation. On the other hand, it draws attention to the comparison of this group with the 30%+Vit.D group, since the difference between these groups has the same significance expressed above (Fig. IV M). Based on this analysis, it is possible to infer that Vit.D did not attenuate the redox imbalance imposed by the ingestion of fructose at the concentrations defined by the study protocol.

According to Busserolles et al. (2003), in an experimental study with rats treated with a high-fructose diet, the data showed an increase in TBARS levels, attributed to the increase in the synthesis of reactive oxygen species, determining pro-oxidant conditions. Rivera-Ramírez et al. (2011) conducted an experimental study using mice submitted to a diet containing 60% fructose administered via gavage. The results showed high rates of TBA-RS, assumed as evidence of lipid peroxidation. Results with levels of TBA-RS, elevated after treatment with 10% fructose solution, and with rats with 30 days of life, pointed out as statistically significant, were presented by (VAZQUEZ-PRIETO et al. 2011). The marked lipid peroxidation in the hepatic tissue can be explained since fructose metabolism is carried out predominantly in the liver and to a lesser extent in the proximal epithelium of the renal tubules, in addition to the small intestine (BARREIROS et al., 2005). Escaping the context of the current study, but relevant to the topic, Girard et al. (2006) showed a reduction in the synthesis of antioxidant elements in the blood in fructose-fed rats.

In renal tissue, the current study did not show increases in TBA-RS levels in the control group compared to the other groups. This aspect shows that, possibly, the renal tissue is less affected by the increase in plasma concentrations of fructose and the compounds generated by its metabolism.

Reactive oxygen species perform several biological functions in addition to the potential to destabilize some aspects of redox homeostasis (DRÖGE, 2002). Differently from what is usually disclosed, these chemicals can exert physiological functions such as initiating calcium signaling transduction cascades, but excess can lead to cell death by lipid peroxidation present in cell membranes and protein oxidation. On the other hand, changes in the redox state, nowadays, are also considered basic mechanisms for the development of chronic non-communicable diseases in humans, such as diabetes and neurodegenerative diseases (PHAM-HUY 2008). According to the same source, the degree of tissue damage varies depending on the composition and properties of the tissue and the balance of oxidative stress and antioxidant defense in the intracellular compartment.

Other researchers such as Lushchak (2014) describe that oxidative stress can be classified according to intensity, through scales, ranging from (eustress) to the toxic oxidative load that damages biomolecules. These statements gain relevance since there is already research indicating that diseases such as type 2 diabetes mellitus may be due, but not exclusively, to redox imbalance (WATSON, 2014). However, a study conducted by Wang et al. (2017), carried out with people with Vit deficiency. D, did not make the link between Vit concentrations clear. D and the redox imbalance. On the other hand, in healthy individuals, the increase in the expression of reactive oxygen species drives, among other mechanisms, the activation of the transcription of the erythrocyte-derived nuclear factor, providing cell signaling and, this way, stimulates the increase in the expression of antioxidant genes (TRACHOOTHAM et al. al., 2009; GORRINI et al., 2013).

Reactive free oxygen species induce lipid peroxidation, being identified as a cause of cellular damage. Lipid peroxides increase platelet aggregation and when the cellular antioxidant mechanism is exhausted, cell membranes can suffer irreversible damage. This and other pathological conditions such as complications in preeclampsia may be associated with redox imbalance (RASHID et al., 2018).

Other studies have shown that excessive fructose intake in animal models caused damage to various tissues associated with increased carbonyl groups and oxidative stress (DONG et al., 2010; YANG et al., 2011). In vitro studies have also shown that fructose generates an increase in reactive oxygen species and reactive carbonyls when compared to glucose (SAKAI et al., 2002).

Since the discovery of oxidants and their potential harm to biological systems, many efforts have been spent to determine their correlations as well as the attempt to establish therapies to neutralize the harmful effect attributed to these substances. Vit.D, due to its biological potential, has also been pointed out for its antioxidant action. On the other hand, all therapy must be based on evidence due to consistent research and failure to comply with these criteria, in addition to not obtaining benefits, may express the dark side of antioxidants, exerting harmful functions for essential organic functions (SARANGARAJAN et al. 2017).

Parthiban et al. (2016); Xu et al. (2017) describe many factors to be considered in the indication of the use of antioxidants as a preventive measure to minimize the risks for the development of chronic non-communicable diseases, referring that therapy with antioxidants can be considered a resource to assimilate the desirable effects of antioxidants. to potentially reduce the risk of developing diseases or prevent their aggravations.

Data from the current study showed that Vit.D showed no potential to attenuate some aspects of oxidative stress in liver and kidney tissue. On the other hand, the reduced bioavailability of, for example, GSH can be attributed to excessive consumption for the maintenance of redox homeostasis. On the other hand, Kund et al. (2011) reported a significant increase in GSH levels in blood and liver samples from mice treated with fructose-sweetened water when compared to the control group. However, Ozdogan et al. (2012) demonstrated that animals supplemented with a high-fructose diet had reduced levels of GSH.

The treatment of redox homeostasis disorders aimed at preventing the deleterious effects of this imbalance, although there are controversies regarding its effectiveness, some studies consider it as a potential strategy to minimize the potential pathological action of these chemical compounds on organs or tissues (BURGOYNE et al, 2012; MOHAMMEDI et al., 2015). However, there are no consensus or consistent studies that ensure any benefit with antioxidant therapies and must be directed to individuals who have increased oxidative stress (ROBINSON et al., 2006).

In this sense, observational studies offer consistency by correlating the association between a low level of Vit. D and the risk of type 2 diabetes (LU et al, 2018). Pittas et al (2019) conducted a randomized, double-blind, placebo-controlled study to assess the safety and efficacy of oral administration of Vit.D (4000 IU per day) for the prevention of diabetes in adults at high risk for type 2 diabetes. Results showed that this therapy did not result in a significantly lower risk of diabetes than placebo.

## CONCLUSIONS

The consumption of fructose in the two proposed concentrations was associated with maternal and fetal disorders, and, based on these results, the study can be defined as relevant and of public health interest.

Taken together, the results showed that the consumption of fructose impaired the fertility of the sows, also determining the reduction of the weight of the offspring, indicating possible toxicity for the pregnancy. The analysis also showed disturbances in lipid, glycemic, hepatic and renal function metabolism, in addition to oxidative stress, becoming more pronounced for the groups in which the fructose concentration was higher and the addition of Vit.D was not efficient to maintain the balance. redox

The repercussion of this dietary pattern was shown to be important on frucundity and that fructose intake can be an important predictor of metabolic risk, directly interfering with some gestational and metabolic parameters.

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