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Research Article

EFFECT OF RESISTANT STARCH FROM HYPOCOTYL AXES ON THE PREVENTION OF OBESITY IN ADULT MALES RAT FED WITH HIGH FRUCTOSIS AND FAT

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A B S T R A C T

A study was carried out to evaluate the effect of resistant starch from hypocotyl axes on the prevention of obesity in adult males rat fed with high fructosis and pig fat. Six groups of six rats each were subjected to this study, in particular a positive control group, a control group and four groups each having in their diet an incorporation of flour from hypocotyl axes in different proportions, especially 5, 10, 15 and 20 %. The results of the analyzes show an increase of 12.34% and 28.51% of the body mass of the control group and the positive control group. A decrease of the body mass in the rats subjected to supplemented diets were observed. The total cholesterol contents were 201.49 ± 4.67 and 242.04 ± 4.53 mg respectively for the control group and the positive control group. From the 5 to 20% concentration of hypocotyls axes flour, a lowering of 14.02% body mass was observed. The values obtained for HDL cholesterol ranged from 43.93 ± 1.07, 70.45 ± 2.14 to 76.01 ± 4.43 mg / dl respectively for the control group, the control group and the group feed at 15 % of hypocotyl axes and those of LDL cholesterol were 157.85 ± 9.16, 176.12 ± 15.10 and 141.68 ± 9.85mg / dl respectively for the control group and the feed group 20% hypocotyl axis. The blood glucose level was 94.17 ± 3.08, 127.50 ± 12.83 and 103.33 ± 8.08 g / dl respectively for the control group, the control group and the group feed 20% hypocotyl axes. The hypocotyl axis can be used for the prevention and management of obesity.

INTRODUCTION

Resistant starch is not well digested in the gastrointestinal tract by enzymes, so that they can be used to prevent obesity (Thompson, 2000). The physical characteristics of gastric and intestinal contents are altered by the presence of resistant starch (Bingham *et al.*, 2003). In the gastrointestinal tract, this starch swells, capturing water and nutrients, in particular water-soluble nutrients, such as soluble sugars and influence gastric emptying, dilute enzymes, substrates and nutrients in the gastrointestinal tract. This slows the passage of nutrients into the blood (Bingham *et al*., 2003; Timm *et al.*, 2013).

Many hypothesis have linked inadequate consumption of dietary fiber, resistant starch and physical inactivity with the occurrence of overweight. Poor diet and physical inactivity are among the main risk factors for non-communicable diseases, which are particularly addressed in the global strategy on diet, physical activity and health (WHO, 2009). Obesity and overweight is now the world's fifth-target for the prevention of mortality. The results for plants rich in resistant starch are limited to conventional foods. Non-timber forest products also have nutritional values just as interesting as known foods. They can be used to prevent and fight against obesity and overweight.

In sub-Saharan Africa, particularly in Cameroon, the hypocotyl axis is highly consumed by the populations from the northern part. Study on physico-chemical hypocotyl axis showed that it contains a high resistant starch (Ali *et al.*, 2010). Form these results, effect of resistant starch from hypocotyl axes on prevention of obesity in adult males rat feed with high fructosis and pig fat was carried out. The purpose of this work is to validate the experimental model through regimes containing different proportions of resistant starch of hypocotyl axes and to obtain the optimal dose of resistant starch exhibiting regulatory effects of metabolism in an obesogenic environment and having the least side effects.

MATERIAL AND METHODS

Plant and animal material

The plant material consists of the hypocotyl axes of *B. aethiopum* obtained from roots cooked at 100 ° C for 15 min and then crushed and sieved to a grain size of ≥50 µm. Adult male Sprague Dawley albino rats of three months of age obtained at the Nutrition Laboratory of the University of Yaounde were used for this experiment. These male adult rats also show a good growth curve and a regular production of hormones compared to females (Harlan, 2004).

Acclimatization, distribution of rats and diets

The rats were weighed and divided into 6 groups and then housed in a single cage at 6 rats per cage. The animals were kept in an environment of 25 ± 2 ° C with a lighting / dark cycle (12h / 12h: 7-19h and 19-7h) (Portillo *et al.*, 2001). These rats were acclimated for one week and fed a standard maintenance diet and water (Diet A04). The composition of the diet used for control group rats is the 210 diet, which is slightly different from the diet commonly used in pet stores (Marion, 2011). The diet 210 ("control" diet) is in fact richer in fat than the standard diet A04 (respectively 13% and 9% of calories are provided by fats). Scheme 210 is marketed as a "control" regimen for HF-Lard and HF-Coco diets. Its composition is such that the amino acid intakes are identical between the High-Fat diets and the "Control" diet. However, its lipid composition makes it a special diet. For the test groups, we chose the diet enriched with lipids and carbohydrates because it allows to approach as closely as possible the new nutritional habits. Table 1 shows the dietary composition of the control diet (control 210) and that of the positive control group: diets enriched in pig fat and fructose.

A portion of the dough obtained is flattened manually to formulate the croquettes of 5 to 6 cm in length, 3 cm in width and about 2 cm in thickness which are dried in a ventilated oven (Memmert oven, Germany) to 50 ° C for 24 hours (Appleford and Anderson, 1997). Table 2 shows the composition of different diets.

Determination of consumption index and body weight gain in rats

The feeding of the rats was carried out for one month, from 05 June to 05 July 2013. The formulated kibbles were served twice a week to the rats according to the method of Gaïva *et al*., (2003).

The quantities of food consumed were evaluated twice a week at the same time (9 am). The masses of the rats were taken on the same days. The changes in the consumption index and body weight gain of rats were calculated as percent consumption and mass gain of each rat, respectively, relative to the feed mass served and the body mass of the rats.

Table 1 Protein, carbohydrate and lipid composition of control diets (control 210), and diets enriched in swine fats and fructose (Marion, 2011).

Ingrédients (%)		Control 210	*Valeur énergie (Kcal/100g)*	Positive control	*Valeur énergie (Kcal/100g)*
Protein	Casein	19	*76*	19	*76*
	L-cystin	0,5	*2*	0,5	*2*
	Corn starch	58	*232*	32,5	*130*
	Fructose			15	*60*
Carbohydrates	cellulose	5	/	5	/
	Total	82,5	*310*	72	*268*
	Oil in bulk	12,5	*112,5*	3	*27*
Lipids	Pig fats			20	*180*
	Total	12,5	*112,5*	23	*207*
Minerals mixture		4		4	
Vitamins (%)		1		1	
Energie	Total	100	*422,5*	100	*475*

Preparation of foods for preventive nutritional analyzes

The diets were formulated from a food containing 19% casein, 0 to 32.5% crushed corn starch fermented overnight, 15% fructose, 20% pig fat, 3% Oil in bulk, 5% cellulose and 5% vitamin and mineral mixture (Kelavital Kela-Belguim Hoogstraten-Belguim) according to the American Institute of Nutrition (AIN) (1977). 0 to 40% of the flour of hypocotyl axes was added. To the formulated flour is added 600 ml of water, the whole is mixed to form a paste.

Blood collection and treatment

The blood of rats fasted for 14 h was taken for general analyzes. It was collected by cardiac puncture after anesthesia with diethyl ether using a syringe and then introduced into the dry tube is allowed to stand for 4 hours for the collection of the serum and finally was centrifuged at 3000 rpm for 5 min. The collection of the serum was done at the same time to avoid any possible variations.

Table 2 Dietary protein, carbohydrate and lipid composition. RS: Resistant Starch, DS: Digestible Starch

Ingrédients (%)			**Témoin positif**	**RS 5%**	**RS 10%**	**RS 15%**	**RS 20%**
Protin		Casein	19	19	19	19	19
		L-cystin	0,5	0,5	0,5	0,5	0,5
		AM	32,5	27,5	17,5	7,5	0
		Fructose	15	15	15	15	15
Glucides	Flour of hypocotyles	AS	/	5	10	15	18,5
		starch	/	5	10	15	19
		Cellulose	5				
	Lipids	Pig fat	20	20	20	20	20
		Oil in bulk	3	3	3	3	3
Mineral mixture			4	4	4	4	4
Vitamins			1	1	1	1	1
E(Kcal)		**Total**	**475**	**465**	**455**	**445**	**435**

Determination of total cholesterol and bound cholesterol (HDL and LDL)

The levels of total cholesterol, HDLc and LDLc were determined by enzymatic methods using kits:Human Cholesterol liquicolor SU-CHOL 10017 (Biochemica und Diagnostica mbH, Germany); Human SU-HDLDD 10084 (Human, Germany) using an enzymatic reagent (R1) and a standard solution (R2) adapted to the Mindray BA-88 analyzers. Human 10094 (Human Germany) consisting of two directly automatable reagents (R1 and R2) (BA-88 Analyzers). Reagent R1 contains a detergent which solubilizes non-LDL lipoproteins. The cholesterol thus released from HDL, VLDL and chylomicrons is converted by cholesterol oxidase and cholesterol esterase into an inactive and colorless product. The R2 reagent contains a second detergent which dissolves the LDL lipoproteins and releases the LDL cholesterol fraction (Roeschlau *et al.*, 1974).

Determination of triglyceride levels

Triglycerides were assayed by the GPO-PAP colorimetric method. This method uses a series of enzymatic reactions coupled using Human SU-TRIMR 10720P kit (Human, Germany). The triglycerides in the sample were hydrolysed by a set of microbial lipases to form glycerol and fatty acids (Fossati and Principe, 1982).

Glucose tolerance test in normal rats and blood glucose testing

Blood glucose was measured using a one touch glucometer using the test strip method of the same brand.

A drop of blood was taken from the tail end of the rat with a self-tapping device and deposited on a glucose oxidase-impregnated test strip. 10 seconds after deposition of the blood drop, the blood glucose value is displayed on the glucose monitor.

Analysis of liver function

Hepatic function is determined by the determination of the levels of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), according to the recommendations of the International Federation of Clinical Chemistry (IFCC). During the reaction, the decrease in the absorbance measured at 340 nm, due to the consumption of NADH, is directly proportional to the activity of the ALAT or ASAT in the sample. Endogenous pyruvate disappears during the incubation period (Bergmeyer, Horder and Rej, 1986). The assay uses the humans kits (EN-GPTU 12212 and EN-GTU 12211 respectively for ALAT and ASAT) adapted on the mindray BA-88 analyzers (Biochemistry Analyzer, Manshan chenzhen 518057 P.R., China).

Determination of creatinine level

The creatinine content was determined by spectrophotometry at 520 nm using the Jaffé method without deproteinisation with the Human Su-Crea 10052 kit (Bartels *et al.*, 1972).

Statistical analysis

The results obtained were an average of six rats and were expressed as averages and standard deviations. The analysis of variance was used to compare the averages. Duncan's multiple comparison test was used to rank averages when there was a significant difference using the Statgraphics Centurion XV.II software. The tool used to draw the curves was the Sigmaplot 11.0 software.

RESULTS AND DISCUSSION

Food consumption index

The food consumption index is shown in Fig 1. There is a variation in the average consumption of foods from one diet to another. This variation results in a decrease in the IC of the rats subjected to the two control regimes and an increase thereof in the rats supplemented with the different proportions of resistant starch. In rats whose consumption indices are high, there is a decrease in their body masses.

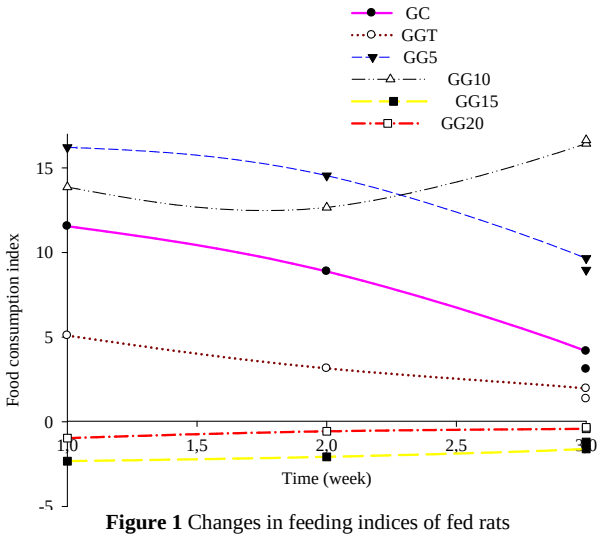


Figure 1 Changes in feeding indices of fed rats

GC: control group consisting of animals subjected to standard feeding;
GGT groups positive control fat, consisting of food-fed animals enriched with animal fats and fructose;
GG5: fat groups 5%, includes animals subjected to a diet enriched in animal fats and fructose with incorporation of 5% of the flour of hypocotyl axes;
GG10: fat groups 10% comprises animals subjected to a diet enriched with animal fats and fructose with incorporation of 10% of the flour of hypocotyl axes;
GG15: fat groups 15% comprises animals subjected to a diet enriched with animal fats and fructose with incorporation of 15% of the flour of hypocotyl axes;
GG20: fat groups 20%, includes animals subjected to a diet enriched in animal fats and fructose with incorporation of 20% of the flour of hypocotyl axes.

Evolution of body mass

Fig. 2 shows the evolution of the body masses in g of the rats subjected to the different regimes during one month of experimentation.

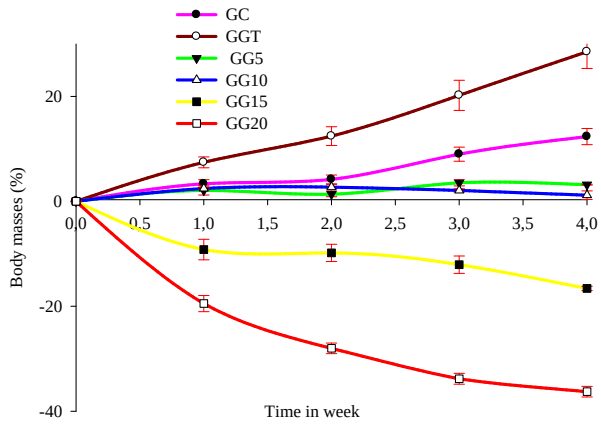


Figure 2 Variation of the masses during the experimental period.

The analysis of the variances shows a significant effect of the consumption of the flour of hypocotyl axes at different threshold concentrations, $p < 0.05$ on the body mass of the rats

in all the groups. The body mass of the animals receiving the control feed and that of the rats of the positive control group increased from the first week. This increase is continuous until the end of the experiment. There was an increase of 12.34% and 28.51% of the body mass of rats after one month of experimentation for the two group's control, respectively. For the groups receiving 5% of the flour= hypocotyl axes, the increase in the body mass of the rats is rather low with a percentage of 3.18%. For the group of rats receiving 10% of the flour of hypocotyl axes their body masses vary little. From 15% incorporation of the flour of hypocotyl axes, the effects of resistant starch were remarkable on the body masses of rats supercharged with fat and fructose, in that a gradual decrease of their body masses from 221.51 ± 1.95 to 184.71 ± 1.65 and 230.16 ± 7.19 to 146.66 ± 5.03 with percentages 16.61% to 36.28% for the groups receiving 15 and 20% of the flour of hypocotyl axes. Concerning the starch of hypocotyl axes, the concentrations having an effect on the decrease in body weight are 15 and 20%.

Total serum cholesterol

The results of analyzes on the percentages of the total serum cholesterol of the rats are presented in Fig. 3. There is, a decrease in the total cholesterol contents of the rats of the groups incorporated in the resistant starch of hypocotyl axes at different concentrations in particular 5, 10, 15 and 20% relative to the rats of the control group. The total cholesterol levels were 201.49 ± 1.67 and 242.04 ± 1.53 mg / dl respectively for the control group and the positive control group. This decrease in total cholesterol is progressive as the concentration of resistant starch of hypocotyl axes increases. From the 5 to 20% concentration, a reduction of about 14.02% was observed.

The HDL cholesterol levels increase in rats in the control group and in groups fed at different concentrations of hypocotyl axes. The values obtained for HDL cholesterol ranged from 43.93 ± 1.07, 70.45 ± 2.14 to 76.01 ± 1.43 mg / dl for the control group, control group and group fed at 15 % of hypocotyl axes. An increase of 76.01% HDL cholesterol from the group of rats whose feed comprises 15% of the flour of hypocotyl axes relative to the control group is observed.

The LDL cholesterol levels of the rats decreased from the control rat group to the rat groups fed at different concentrations of hypocotyl axes. The values obtained for LDL cholesterol were 157.85 ± 1.16, 176.12 ± 1.10 and 141.68 ± 9.85mg / dl respectively for the control group and the group fed at 20% d Hypocotyl axes. There was a statistically significant difference between groups at the 95.0% confidence level.

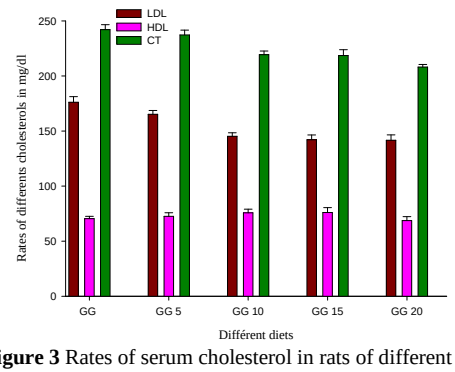
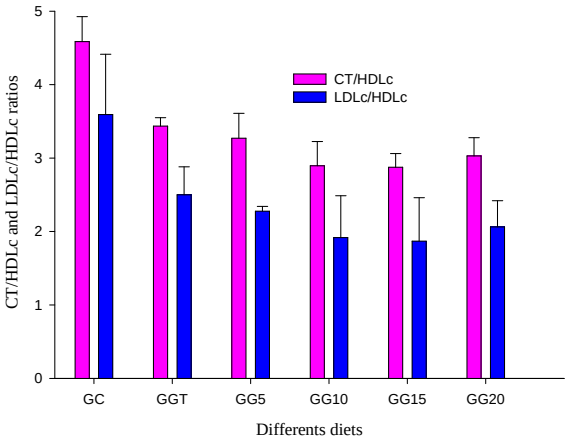


Figure 3 Rates of serum cholesterol in rats of different diets

Risk Markers for Cardiovascular Disease and Coronary Heart Disease

The LDLc / HDLc ratio is linked to a risk factor for coronary heart disease, the risk increases as this ratio. This risk is low when LDLc / HDLc <3.5. From the observations made in FIG. 4, it appears that neither the control groups nor the groups having received the resistant starch of hypocotyl axes present this risk whatever the concentration used. The ratios obtained range from 1.86; 1.91; 2.06; 2.27 and 3.59, respectively, of the groups of rats fed at 15, 10, 20, and 5.0 % resistant starch and the control group.



Serum triglyceride levels

The triglycerides' levels of rats in the control groups decreased compared to rats in feeding groups with incorporation of increasing concentrations of hypocotyl axes. Their values fluctuated between 193.33 ± 2.04 ± 176.26 ± 1.36 and ± 174.66 ± 1.19 mg / dl respectively for the positive control group, the control group, and the group fed 20% hypocotyl axes. There was a statistically significant difference between groups at the 95.0% confidence level. There is an increase in the order of 9.68; 6.20; 4.08 and 3.6347%, respectively, for the positive control group and the 5, 10 and 15% hypocotyl axis groups.

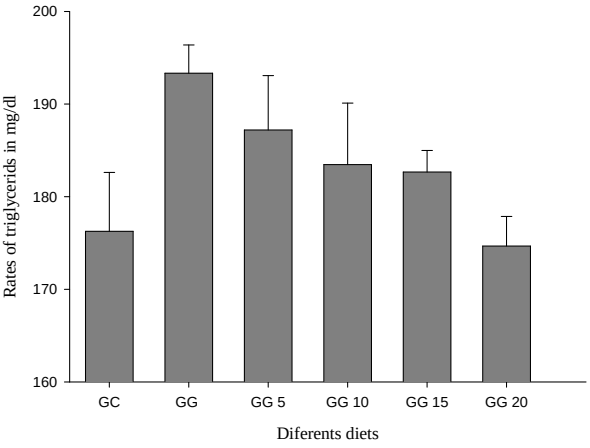


Figure 5 Triglyceride levels of rats of different diets.

Blood sugar levels

The blood glucose values were 94.17 ± 1.08, 127.50 ± 2.83 and 103.33 ± 2.08 g / dl respectively for the control group, the

control group and the group fed 20% Hypocotyl axes. The percentages of increase in blood glucose relative to the control group were 35.39; 30.40; 13.62; 10.97 and 9.73% respectively for the control group and groups fed at 5, 10, 15 and 20% hypocotyl axes. There was a statistically significant difference between groups at the 95.0% confidence level.

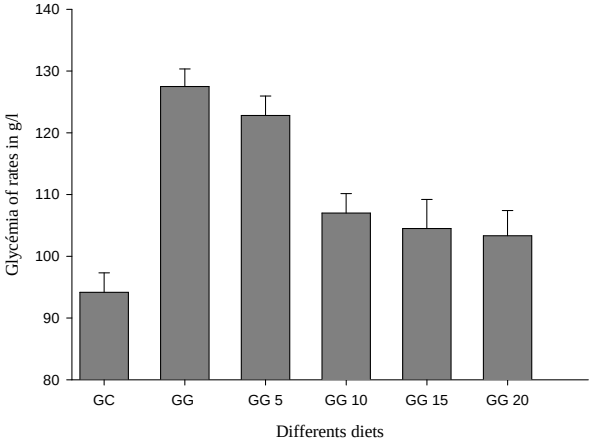


Figure 6 Glycemia of rats under different regimens.

Glucose Tolerance Test

The fig. 7 shows the glucose tolerance test of the flour of hypocotyl axes. The glucose levels of the rats of the different groups increase. The optimum of the control group is reached after 30 minutes while that of the positive control group is at 40 minutes and finally that of the test group at 60 minutes. At the optimum, there is a decrease in blood glucose levels in the different groups.

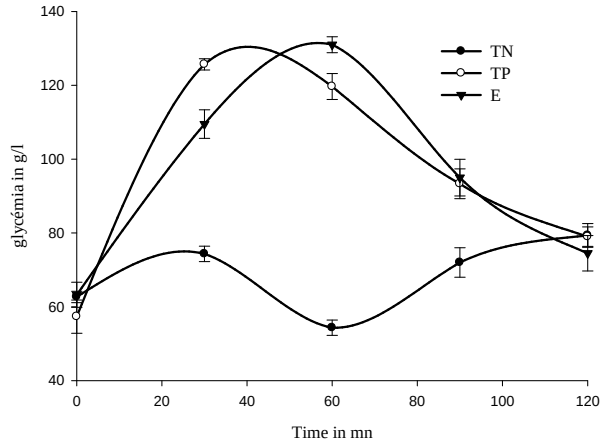


Figure 7 Glucose tolerance test of the flour of hypocotyl axes

TN: Negative control group receiving only water; TP: Positive control group receiving water and glucose solution at a dose of 2 g / Kg. PC; E: Test group (Assay) receiving the extract at the dose of 400 mg / Kg. PC and the glucose solution at a dose of 2 g / kg. PC.

Rate of ASAT ALAT transaminases and creatinin

The ASAT values were 33.17 ± 1.49, 38.35 ± 0.62 and 41.48 ± 0.29 IU respectively for the control group, control group and group fed at 15% Hypocotyl axes. Values for ALAT were 15.78 ± 3.59, 29.68 ± 2.46 and 38.41 ± 4.93 IU respectively for the control group, control group and group fed at 20% Hypocotyl axes. There were statistically significant differences between groups at the 95.0% confidence level.

Creatinine levels values range from 12.08 ± 0.35, 12.66 ± 0.94; 9.33 ± 0.19 and 11.16 ± 0.23 IU respectively for the control group, the positive control group, the groups receiving 15% and 20% hypocotyl axes. There were statistically significant differences between groups at the 95.0% confidence level.

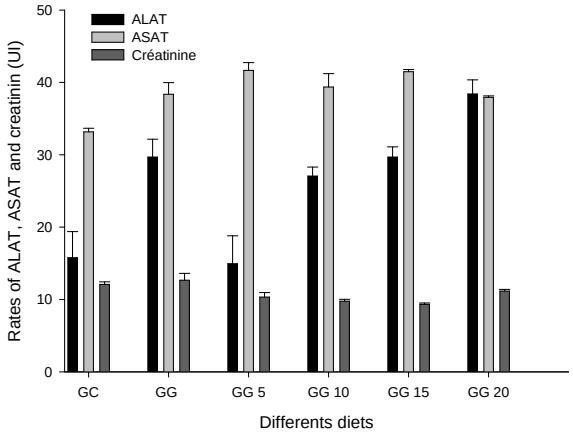


Figure 8 Assessment of hepatic and renal function of fed rats
Incorporation of rice-resistant starch into the diet does not adversely affect renal and hepatic function in rats.

DISCUSSION

The measurement of the body mass of the rats in this study makes it possible to judge the impact of the supplementation of the resistant starch of the hypocotyl axes. Today, dietary habits have changed, the tendency is much more oriented towards the abandonment of traditional foods based on cereals and tubers very rich in resistant starch, fibers and antioxidants, for foods increasingly hypercaloric rich in fats and simple sugars (Hester, 2011). Resistant starch releases compounds in the colon during the fermentation that they have trapped in the small intestine, such as bile salts and fatty acids that are capable of increasing colon secretion and recto traction sigmoid (Timm, *et al.*, 2013).

Carbohydrate-rich meals are usually larger than lipid-rich meals, and carbohydrates satisfy more than lipids, with equal energy loads. The storage of carbohydrates in the body in the form of glycogen is limited. When the reserves are met, the body adapts to oxidize any excess carbohydrates. On the contrary, dietary lipid reserves are unlimited, and the body adapts only extremely slowly to oxidize excess lipids (Manson *et al*., 2004).

The hypocholesterolemic effect due to the consumption of the flour of hypocotyl axes could be justified by the sequestration of dietary lipids in the gel of resistant starches, with reduction of phospholipids available to disperse food cholesterol which hinders its assimilation. The bile salts have their excretion increased, which obliges part of the endogenous cholesterol to enter the pool of bile acids to compensate for these losses.

One of the volatile fatty acids produced in the colonic propionic acid plays a role in regulating the endogenous synthesis of cholesterol. Some authors believe that the beneficial effect on cholesterol is also the consequence on carbohydrate metabolism (Delzenne, Neyrinck and Cani, 2012). HDL-cholesterol is a so-called high-density lipoprotein. Insofar as it intervenes in the elimination of cholesterol, it is also called "good cholesterol". Its increase is

considered a protective factor of cardiovascular risk and there is an inverse relationship between the concentration of HDL-cholesterol and the frequency of cardiovascular complications. The determination of HDL cholesterol is an element of atheromatous risk assessment when there is an imbalance in the CT / HDL or LDL / HDL ratios (Funes, Cado, Cuvelier, Fléchet, Hamida, Huguet *et al*., 1995).

The positive effects of resistant starches on cardiovascular health were related to decrease systolic and diastolic blood pressure, blood cholesterol and triglycerides (Mitchell, 2013). The influence of resistant starches on the absorption of nutrients, especially on cholesterol and triglycerides, allowing a better cardiovascular health.

To be absorbed, nutrients must move from the lumen to the intestinal wall where they will be in contact with the enterocytes. However, prior to this, molecules must also pass through the viscous layer of mucus formed by the food bolus and the enterocytes (Dikeman and Fahey, 2006). The addition of resistant starch increases the viscosity and promotes laminar regime, which reduces the convective movement of particles and creates a layer of thicker mucus. If the bolus food is more liquid, the layer of mucus will be finer which will favor a turbulent regime. The nutrients will then be more favorably stirred centrifugally and will be in contact with the intestinal epithelium which they can then cross (Lentle and Janssen, 2008).

The ingestion of the resistant starch is accompanied by a decrease in the absorption of nutrients by complexations in the gel formed. On the other hand, fermentation of resistant starch increases the number of intestinal L-cells and their secretion products - GLP-1 and PYY - both involved in the regulation of carbohydrate homeostasis and satiety. GLP-1: glucagon-like peptide-1; PYY: YY peptide (Nathalie, Delzenne, Patrice and Cani, 2008). Indeed, ingestion of resistant starch increases the endogenous production of peptides such as glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic peptide (GIP) and YY peptide, and decreases plasma Ghrelin, an orexigenic hormone. The products of bacterial catabolism of starch such as butyrate are involved in increasing the production of GLP-1 by endocrine colon cells (Nathalie, *et al*., 2008).

This study confirms the hypothesis on the beneficial effects of the consumption of resistant starch on the management of the body weight in the context of a diet enriched with fats and fructose. The targets of action of resistant starch could be declined to the interconnected levels of insulin sensitivity and lipid metabolism. Resistant starches can affect the process of lipid emulsification in the stomach and small intestine as well as lipase activity and the actual absorption stage. In animals, decreases in dietary lipid and cholesterol absorption have been reported in the presence of resistant starch (Bingham *et al*., 2003).

CONCLUSION

This study evaluated the effect of resistant starch consumption of *B aethiopum* hypocotyl axes on some weight and biochemical parameters of rats. This study shows that resistant starch significantly decreases the body mass of rats. The hypocholesterolemic and hypoglycaemic effects of this starch were noted. The decline in triglyceridemia was also observed in this experiment. The percentage of incorporation of the most appropriate hypocotyl axis meal is 15% in view of the improvement in HDL cholesterol obtained and the decrease in body mass.

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